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Cover photo: A crab spider, *Tmarus* sp. (Thomisidae), from Singapore. Illumination with ultra-violet light causes its prosoma, legs, and pedipalps to fluoresce a brilliant blue. Photo by Nicky Bay (sgmacro.blogspot.com).

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Revision of the Nearctic *Eratigena* and *Tegenaria* species (Araneae: Agelenidae)

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Abstract. Based on specimens from several museum collections and recently sampled spiders during a field excursion to Mexico in 2014, the 11 species of *Tegenaria* s. l. endemic to the United States of America and Mexico are revised. Morphological characters and mitochondrial DNA sequences (CO1, NADH1, 16S) serve as the basis for proposed new combinations and new species. *Tegenaria chiricahuae* Roth, 1968 remains the only endemic *Tegenaria* species in the Western Hemisphere. All other specific names (*T. blanda* Gertsch, 1971, *T. caverna* Gertsch, 1971, *T. decora* Gertsch, 1971, *T. flexuosa* F.O. Pickard-Cambridge, 1902, *T. florea* Brignoli, 1974, *T. gertschi* Roth, 1968, *T. mexicana* Roth, 1968, *T. rothi* Gertsch, 1971, *T. selva* Roth, 1968, and *T. tlaxcala* Roth, 1968) are transferred to the genus *Eratigena* Bolzern, Burckhardt & Hänggi, 2013. Six new species are described: *E. edmundoi*, *E. feruandoi*, *E. guanato*, *E. queretaro*, *E. xilitla*, and *E. yarini*. In addition, females of *E. flexuosa*, and *E. gertschi*, and the male of *E. florea* are described for the first time. A phylogeny based on maximum likelihood analysis of combined mtDNA sequences, an identification key and images of all diagnosed species are provided.

Keywords: *Eratigena*, mtDNA, new combination, new species, morphology

http://zoobank.org/?lsid=urn:lsid:zoobank.org:pub:4F518AA0-7745-403A-9EDF-A84622E9BEB7

Among the 114 known spider families, the Agelenidae comprises more than 1,160 described species and ranks as the 11th most diverse group (World Spider Catalog 2015). Due to the obvious funnel-webs produced by many species, some of them are well known: for example, the large, long-legged European house spider (*Eratigena atrica* (C.L. Koch, 1843), formerly *Tegenaria atrica*), or the American grass spiders (*Agelenopsis* Giebel, 1869; 13 species). However, new species are still being discovered frequently (Bolzern & Hervé 2010; Bolzern et al. 2009, 2013a, b; Bosmans 2011; Maya-Morales & Jiménez 2013). Our knowledge of the taxonomy and phylogeny of this spider group has fundamentally improved in reeent years (Bolzern et al. 2010, 2013a; Miller et al. 2010), and currently the family can be divided into two subfamilies. Ageleninae generally shows a Holarctic distribution, but includes five genera found only in the Afrotropical (two genera) or the Neotropical (three genera) region. One of these genera was first described in 2013 with six new species and is only known from the Baja California peninsula in Mexico (Maya-Morales & Jiménez 2013). The seeond subfamily, Coelotinae, forms a Holarctic lineage most diverse in Asia, with only two genera exclusively in North America.

The subfamily Ageleninae includes the genus *Tegenaria* s. l., composed of species endemic to the Palearctic or Nearctic regions. The European representatives of this genus were recently revised and grouped in four monophyletic genera (Bolzern et al. 2010, 2013a): *Aterigena* Bolzern, Hänggi & Burckhardt, 2010, *Eratigena* Bolzern, Burckhardt & Hänggi, 2013, *Malthonica* Simon, 1898, and *Tegenaria* Latreille, 1804. The generic affiliation of the 16 Nearctic species is resolved only for the presumably introduced European species, but not for the 11 endemic species. Roth (1952) published the first revision of *Tegenaria* in North America. His original hypothesis, that all *Tegenaria* s. l. species were introduced into the Western Hemisphere from Europe (Roth 1956), was refuted after the discovery of endemic species from Mexico and Arizona (Roth 1968). After that, additional endemic species were described by Gertsch (1971) and Brignoli (1974). In his work, Brignoli noted that this species-complex was extremely problematic due to a lack of images, the very close relationships of the involved species and a lack of diagnostic features. Furthermore, until now, four species were described by one sex only. In view of these complexities and the high proportion of introduced species, a taxonomic clarification is essential for further research or nature conservation approaches.

Therefore, the aims of this paper are threefold: firstly, the taxonomical clarification of the endemic Nearctic and Neotropical *Tegenaria* s. l. species and the publication of images of all endemic taxa; secondly, the description of newly discovered forms or species; and finally, the provision of mitochondrial gene sequences for certain species.

METHODS

Sampling and material examined.—Type specimens (including all type material) and additional material mentioned below were examined from the following institutions: American Museum of Natural History, New York, United States (AMNH: Norman Platnick, Lorenzo Prendini, Luis Sorkin); Biología Comparada, Taxonomía y Sistemática de Araneomorphae, Universidad Nacional Autónoma de México, Mexico (FC-UNAM: Fernando Alvarez Padilla); Colección Nacional de Arácnidos, Instituto de Biologia, Universidad Nacional Autónoma de México, Mexico (CNAN: Oscar Francke, Diego A. Barrales); Museo Civico di Storia Naturale, Verona, Italy (MCSNV: Roberta Salmaso); Muséum National d'Histoire naturelle, Paris, France (MNHN: Christine Rollard); Naturhistorisches Museum Basel, Switzerland (NMB); and The Natural History Museum, London, Great Britain (NHM: Janet Beccaloni). To all specimens examined (excluding existing type material), an identifier was added to the vial (e.g., AB1234). Newly collected specimens are shared between FC-UNAM and the NMB. For

the collection at the NMB, official collection numbers are provided (e.g., NMB-ARAN-12345). Barcoding sequences (CO1 and NADH1) are referenced to the adequate specimens by providing the GenBank accession-number following the identifier.

In addition to the museum collections, specimens were sampled during a field excursion to Mexico in October 2014 (A. Bolzern and E. González Santillán). All specimens were collected by hand and transferred directly into pure ethanol.

Distribution maps or single references of all georeferenced specimens are available on the scratchpads platform, online at http://agelenidsoftheworld.myspecies.info/specimen_observation. In addition, downloadable high resolution images of representatives of the included speeies are available on the same website (Bolzern 2014).

Molecular methods and analyses.—For DNA extractions, two legs were removed from a freshly sampled and alcohol-fixed (pure ethanol) specimen. The ethanol was removed by incubating the cut tissue at 56°C for 10 min. Then the leg was processed according to the protocol for the purification of total DNA from animal tissues (Spin-Column protocol) using the DNeasy Blood & Tissue Kit (Qiagen). The DNA concentration of the resulting solution was measured using the fluorescent dye Picogreen and a Spectrofluorometer. Polymerase chain reaction (PCR) amplifieation of two loci was undertaken by using the following primer pairs: LCO1490 (Folmer et al. 1994) and C1-N-2191 (Simon et al. 1994) for the mitochondrial cytochrome c oxidase subunit 1 gene (CO1, 678 bp), and TL-1-N-12718 (Hedin & Maddison 2001; numbered following Simon et al. 1994) and M510 (Murphy et al. 2006) for the mitochondrial nicotinamide adenine dinucleotide dehydrogenase subunit 1 (NADH1, 591 bp). For PCR, the Qiagen Hotstar polymerase reagents (Qiagen, Germany) were used. The following thermo cycling conditions were applied: initial denaturation step of 95°C for 15 min, followed by 15 touchdown cycles of: 94°C for 35 s, an annealing temperature of 60°C to 45°C for 90 s, and an extension temperature of 72°C for 90 s. After the touchdown cycling 30 additional cycles were added at 94°C for 35 s, 50°C for 90 s, and 72°C for 90 s. Finally, the cycling was followed by an additional extension of 72°C for 5 min. To eliminate incorporated nucleosides and primers, the PCR produets were treated with ExoSAP-IT (GE Healthcare). The fragments were then sequenced in both directions using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Sequences were then analyzed using an ABI Prism 3730 Genetic Analyzer.

Each sequence was proof-read by ehecking the chromatograms by eye using the software FinchTV v. 1.4 (Geospiza Inc.). The complementary sequences (5′ and 3′ directions) of each specimen were aligned using ClustalW 2 (Larkin et al. 2007) on the EBI website (Li et al. 2015) to test the sequence quality. Eaeh sequence was checked for contamination by using the 'Basic Local Alignment Search Tool' (BLAST) on the NCBI website. The alignments of the mitochondrial gene regions were carried out manually, using the translation to amino acids as a guide and checking for any inappropriately placed stop codons and insertions or deletions. All sequenees were then cut to a length of 678 bp (CO1) or 591 bp (NADH1). Within these two alignments no indels or stop codons occurred.

In addition to the protein coding markers, GenBank was searched for 16S sequences of already ineluded taxa. In favor of repeatability and objectivity, the 39 adequate sequences were aligned by using a fixed automatic alignment instead of manually edited alignments or alignments based on secondary structures. Therefore, multiple sequence alignments were earried out using the software package Opal (Wheeler & Kececioglu 2007) implemented in Mesquite (Maddison & Maddison 2015), applying the default parameters (A<–>G: 56; C<–>T: 53; transversions: 100; gap costs: open: 260; terminal open: 100; extension: 69; terminal extension: 66).

The three alignments were eoncatenated using Mesquite, resulting in an alignment eomprising 103 taxa and 1697 bp, and with an overall coverage of 68% (CO1: 101 seq.; NADH: 70 seq.; 16S: 39 seq.). Details are available as Supplemental S1, a list of included taxonomic units for the molecular analysis with GenBank accession-numbers (online at http://dx.doi.org/10.1636/R15-81.s1), and Supplemental S2, a PHYLIP file showing the alignment of the mitochondrial CO1, NADH1 and 16S sequences (online at http://dx.doi.org/10.1636/R15-81.s2). Both files are also available online at http://agelenidsoftheworld.myspecies.info/content/downloads.

Maximum likelihood analysis and bootstrap runs were performed using GARLI 2.01 (Zwickl 2006) at the CIPRES Science Gateway (M.A. Miller et al. 2010). For the two mitochondrial partitions, the codon model was applied, for the 16S partition a GTR+G+I model was used as suggested by the model search function in MEGA 6.0 (Tamura et al. 2013). Bootstrap values were subsequently drawn on the best ML tree using the program SumTrees within DendroPy (Sukumaran & Holder 2010, 2015). Parsimony analysis was performed in TNT Version 1.1 (Goloboff et al. 2008) applying a heuristic tree search with TBR, implied weighting (K=10), and 1000 random additions of taxa while holding 100 trees per iteration. Braneh support was estimated by applying a jackknife resampling method (1000 replicates) with default removal probability of characters (0.36). For both phylogenetic analyses, *Amaurobius ferox* (Walckenaer, 1830) (Amaurobiidae) was used as the outgroup, and resulting trees were rooted at the *Amaurobius* branch/clade.

Morphological methods and abbreviations.—Preserved specimens were examined under a Leica MZ12 and a Leica M165 C microscope. Images were taken using a Leiea MC170 HD camera attached to the Leica M165 C, and processed with the stacking program CombineZP (Alan Hadley) and Adobe Photoshop. To remove soft tissue, dissected female genitalia were first transferred to distilled water for several hours. Subsequently, they were put in an enzymatic lens cleaner solution overnight, washed, and transferred back to ethanol. The morphological terminology follows Bolzern et al. (2013a). The following abbreviations are used (see also Figs. 8, 9, 11, 14–16, 18–20, 22, 27, 28): AER, anterior eye row; ALE, anterior lateral eyes; ALS, anterior lateral spinnerets; AME, anterior median eyes; bulbL, distance of the cymbium base to the most distal tip of the male bulb (including conductor); C, conductor; CB, cymbium breadth; CD, copulatory duct; CL, carapace length; CLY1, clypeus height under AME; CLY2, clypeus height under ALE; CO, copulatory opening at female

epigyne; CW, carapace width; DB, dorsal branch of RTA; DP, distal portion of conductor; DS, distal sclerite at MA; E, embolus; FD, fertilization duct; MA, median apophysis of male bulb; OL, opisthosoma length; OW, opisthosoma width; PER, posterior eye row; PLE, posterior lateral eyes; PLS, posterior lateral spinnerets; PM, posterior membrane (internal posterior limit of female genital area); PME, posterior median eyes; PMS, posterior median spinnerets; PS, epigynal posterior sclerite; PT, epigynal 'pseudo teeth'; R, retroventral ridge of palpal tibia; RC, receptaculum; RTA, retrolateral tibial apophysis (used here as the sum of all structures on the retrolateral aspect of the tibia of the male pedipalp); STL, sternum length; STW, sternum width; T, tegulum; TR, transversal ridge at conductor; VB, ventral branch of RTA.

Taxonomical nomenclature follows the World Spider Catalog (2015).

RESULTS

Molecular data.—Maximum likelihood and parsimony (MP) analyses resulted in essentially identical best trees (Fig. 1, MP tree not shown). The higher level classification proposed by Bolzern et al. (2013a) is supported by the molecular data presented here and summarized as follows (see also Fig. 1): (1), Agelenidae is split into the two monophyletic subfamilies Ageleninae and Coelotinae; (2), the genera *Tegenaria* and *Eratigena* are separate monophyletic clades. However, the relationships between genera are unclear due to low supporting values. Based on mitochondrial DNA, the Mexican-clade represents a monophyletic sister-group to all other included *Eratigena* species (Fig. 1). Within this Mexican-clade, *E. yarini* sp. nov. and *E. ednundoi* sp. nov. represent a closely related, well supported monophyletic subgroup, the *flexuosa*-group. Within the remaining species of the Mexican-clade, the species *E. mexicana* (Roth, 1968) and *E. tlaxcala* (Roth, 1968) are very closely related. A *mexicana*-group, as suggested by morphological data, is not supported by mtDNA data.

Morphological data.—The finding that the Mexican species previously described in *Tegenaria* s. l. are closely related to Palearctic *Eratigena* species is supported by the following morphological characters, which match the genus definition of *Eratigena* Bolzern et al. (2013a): (1), dentition of the retromargin of the chelicerae (six or more teeth, decreasing in size proximally); (2), the PMS bearing one conspicuously prominent spigot; (3), colulus developed as a trapezoidal plate with distal margin w-shaped; (4), absence of a retroventral ridge on male palpal tibia; (5), presence of a transverse hyaline (lamelliform) ridge on the conductor; and (6), presence of appendages at the genital ducts in females (*mexicana*-group). Therefore, all Mexican *Tegenaria* species are here transferred from *Tegenaria* to *Eratigena*.

Based on genital morphology (for details see Taxonomy section), the Mexican *Eratigena* species can be divided into a southern (the *flexuosa*-group, also supported by mtDNA) and a northern species group (the *mexicana*-group).

Tegenaria chiricahuae Roth, 1968, the only species exclusively known from Northern America (USA), differs morphologically from the Mexican species in all characters mentioned above and matches the definition of *Tegenaria*. Therefore, it remains the single endemic representative of the genus *Tegenaria* in the Western Hemisphere.

SYSTEMATICS

Family Agelenidae C.L. Koch, 1837
Genus *Tegenaria* Latreille, 1844

Tegenaria Latreille, 1844: 134. Full synonymy: see World Spider Catalog (2015).

Type species.—*Araneus domesticus* Clerck, 1757, by subsequent designation of Kluge (2007).

Diagnosis.—A detailed diagnosis for the genus *Tegenaria* was provided by Bolzern et al. (2013a: 774–775).

Distribution.—The currently 104 described species (World Spider Catalog 2015; this publication) are primarily distributed in the Mediterranean Region, spreading towards Asia. *Tegeneria domestica* (Clerck, 1757), *T. pagana* C. L. Koch, 1840 and *T. parietina* (Fourcroy, 1785) expanded their distribution areas extensively, most likely due to introductions by humans. In the Western Hemisphere, *T. chiricahuae* represents the only known endemic species.

Tegenaria chiricahuae Roth, 1968
Figs. 8–16

Tegenaria chiricahuae Roth, 1968: 7, figs. 9–11.

Type material.—*Holotype male*. UNITED STATES: *Arizona*: Cochise Co., Chiricahua Mountains, Cave Creek Canyon, 4.83 km W. of Portal, 28 November 1963, V. Roth (AMNH).

Paratypes. UNITED STATES: *Arizona*: 1 ♀ allotype, same data as holotype except 30 June 1963 (AMNH); 1 ♀, same data except 30 November 1962 (MNHN).

Other material examined.—UNITED STATES: *Arizona*: 1 ♀, Cochise Co., Chiricahua Mountains, Cave Creek Canyon, small cave 3.22 km W. of Portal, 2 June 1972, G. Dingerkus (AMNH: AB1155); 1 ♂, 1 ♀, Huachuca Mountains, Carr Canyon, 1829 m, in cave with some litter, 23 March 1964, L. La Pré, M. Eells (AMNH: AB1180). *New Mexico*: 1 ♂, "new cave", 18 December 1976, P. Strinati (MSCNV); 1 ♂, 4 ♀, Eddy Co., Carlsbad Caverns National Park, Midnight Canyon, Ringtail Cave (Flea Cave), 26 May 1973, Wm. Elliott, W.C. Welbourn (AMNH: AB1150); 1 ♂, 1 ♀, same data except Arch Cave, 27 November 1975, W.C. Welbourn (AMNH: AB1121); 1 ♂, same data except Dome Cave, 15 February 1975 (AMNH: AB1149); 2 ♀, same data except Helen's Cave, 31 August 1974 (AMNH: AB1152); 1 ♂, same data except cave, goat trap, 19 February 1976 (AMNH). *Texas*: 2 ♀, Culberson Co., Guadalupe Mountains National Park, cave, upper sloth, 17 April 1976, W.C. Welbourn (AMNH).

Diagnosis.—Male *Tegenaria chiricahuae* can be separated from all other *Tegenaria* species by the simple RTA (Figs. 13, 16), the large median apophysis with a pocket-like distal sclerite and the distally keel-shaped conductor tip (Figs. 14, 15). Females can be distinguished from other species by the distinct conformation of the epigyne and vulva (Figs. 8–12).

Description.—Essential information was provided by Roth (1968).

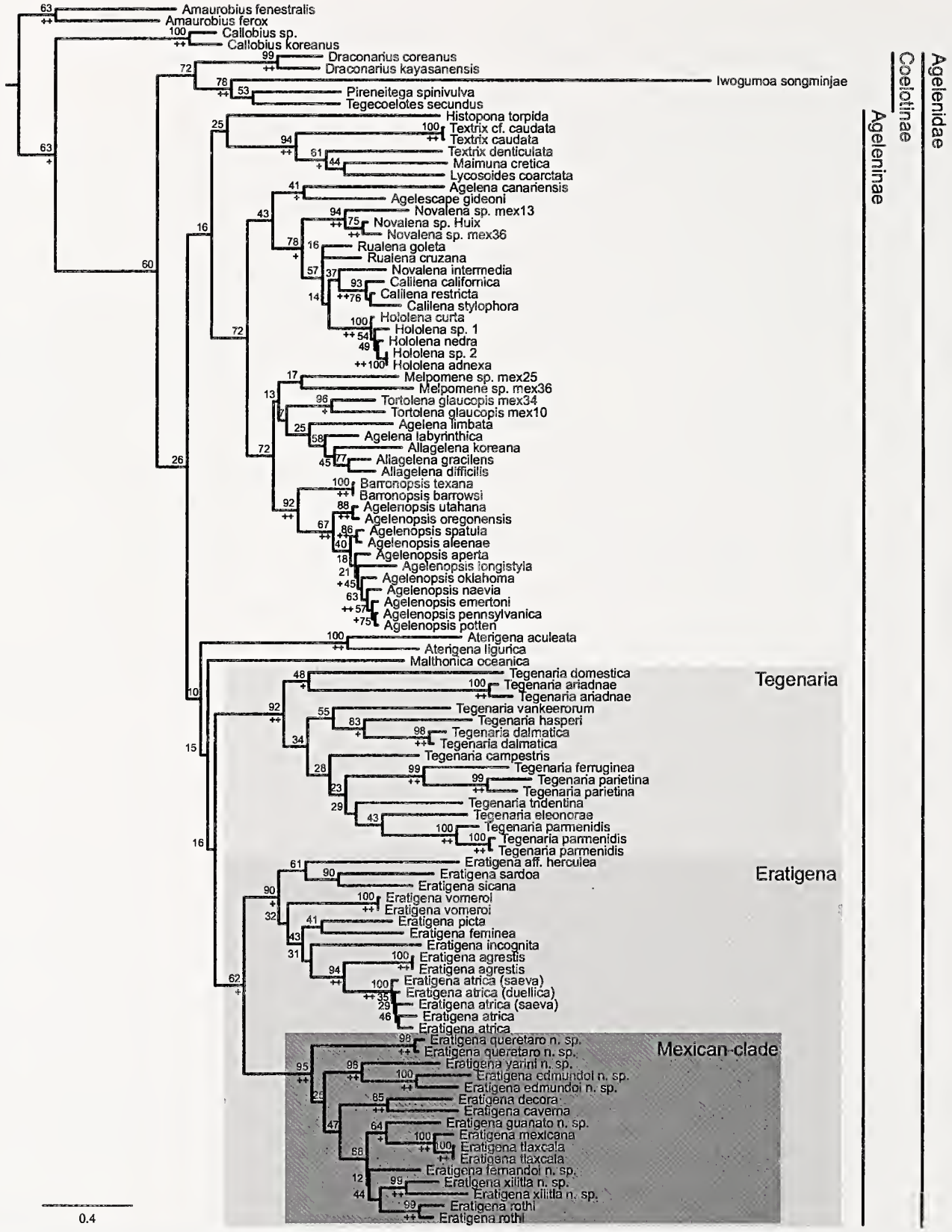

Figure 1.—Best maximum likelihood tree of mitochondrial genes (CO1, NADH, 16S). Bootstrap values are given left and above nodes. Clades with jackknife support higher than 50 % (+) or 85 % (++) from maximum parsimony analysis of the same alignment are indicated left and below nodes.

Distribution.—Reported from several caves in Arizona and New Mexico (United States).

Tegenaria domestica (Clerck, 1757)

Araneus domesticus Clerck, 1757: 76–79, pl. 2, tab. 9, figs. 1–4 (in part). Full synonymy: see Roth (1968) and Bolzern et al. (2013a).

Diagnosis.—Male *Tegenaria domestica* can be separated from all other *Tegenaria* species by the distinct RTA (Roth 1968: figs. 14, 15), the truncated terminal end of the embolus, and the terminally bifid conductor (Bolzern et al. 2013a: fig. 16 W, X). Females differ in having a strongly sclerotized posterior sclerite with the anterior margin concave in combination with a simple, subglobular vulva (Bolzern et al. 2013a: figs. 2 f, 18 b, c).

Distribution.—Introduced from Europe. Cosmopolitan (synanthropic species).

Tegenaria pagana C.L. Koch, 1840

Tegenaria pagana C.L. Koch, 1840: 31, pl. 262, figs. 612, 613. Full synonymy: see Roth (1968) and Bolzern et al. (2013a).

Diagnosis.—Male *Tegenaria pagana* can be separated from all other *Tegenaria* species by the transversally arranged conductor, the elongated finger-shaped distal sclerite of the MA, and the distinet RTA (Roth 1968: figs. 30, 31; Bolzern et al. 2013a: fig. 28 K–L). Females show a high variability in epigynal morphology but differ from others in having a protruding suboval median plate with distinct anterolateral pockets, and a distinct triple twisted vulva (Bolzern et al. 2013a: figs. 28 P–W).

Distribution.—Europe to Central Asia, introduced to North- and South America and New Zealand.

Tegenaria parietina (Fourcroy, 1785)

Aranea parietina Fourcroy, 1785: 533. Full synonymy: see Bolzern et al. (2013a).

Diagnosis.—*Tegenaria parietina* specimens are most similar to specimens of *Tegenaria ferruginea* (Panzer, 1804) but differ from all other species in having a unique RTA and a very strongly U-shaped anterior margin of the posterior sclerite in females (Bolzern et al. 2013a: fig. 21 N–R). Males can be separated from *T. ferruginea* by the much shorter conductor, females by the much less convoluted vulva.

Distribution.—Introduced from Europe. In the Western Hemisphere, reported from the West Indies to Argentina.

Genus *Eratigena* Bolzern, Burckhardt & Hänggi, 2013

Eratigena Bolzern, Burckhardt & Hänggi, 2013: 738

Type Species.—*Tegenaria atrica* C. L. Koch, 1843, by original designation.

Diagnosis.—A detailed diagnosis for the genus *Eratigena* was provided by Bolzern et al. (2013a: 738–741).

Distribution.—The currently 34 described species (World Spider Catalog 2015; this publication) are primarily distributed in the West Mediterranean Region, but with representatives reaching as far east as Laos and 15 endemic species in Mexico. *Eratigena atrica* (C. L. Koch, 1843) and *E. agrestis* (Walckenaer, 1802) were introduced into Northern America.

KEY TO THE NEARCTIC SPECIES OF *ERATIGENA*

1 male palpal tibia with short dorsal spike, median apophysis pocket-like; female vulva with strongly convoluted and enclosed duct 2
- male palpal tibia without short dorsal spike, median apophysis protruding; female vulva with duct not enclosed 3
2 male with massive and broad conductor with strongly sclerotized, pointed terminal appendages; female epigyne with protruding posterior sclerite and deep anterior cavity ..... *agrestis*
- male with strong conductor, terminally with elongated tip; female epigyne with large median area without protuberance or cavity ..... *atrica*
3 male conductor distally distinctly elongated (Figs. 27, 34, 52, 64); female with flattened or coiled copulatory duct without appendages (Figs. 19, 41, 45, 61) ..... *flexuosa*-group, 4
- distal portion of male conductor not distinctly elongated (Figs. 79, 82, 94, 112, 136, 142, 160, 166, 175, 187, 196, 208); female with short and straight copulatory duct with appendages (Figs. 73, 87, 101, 124, 133, 147, 149, 158, 185, 192, 206, 218) ..... *mexicana*-group, 7
4 carapace length smaller than 3 mm; subtegular sperm duct of male bulb c-shaped (Fig. 64); copulatory duct only flattened, not coiled (Fig. 61) ..... *yarini*
- carapace longer than 3 mm; subtegular sperm duct of male bulb moderately s-shaped (Figs. 27, 34, 52); copulatory duct coiled (Figs. 19, 41, 43) 5
5 tegular sperm duct strongly undulated (arrow in Fig. 34); epigynal atrium bordered by pronounced broad ridge (Fig. 38) ..... *flexuosa*
- tegular sperm duct almost straight (Figs. 27, 52); epigynal atrium not bordered by protruding ridge (Figs. 18, 42) 6
6 median apophysis long, with long triangular distal sclerite (Figs. 26, 28); copulatory duct with only one coil (Fig. 19) ..... *edmundoi*
- median apophysis very short, with reduced distal sclerite (arrows in Figs. 51–53); copulatory duct with two coils (Figs. 43–56) ..... *florea*
7 carapaee longer than 5 mm; legs exceptionally long (patella-tibia length leg I > 9 mm); indistinct abdominal pattern, grayish ..... *selva*
- carapace shorter, if equally long, legs shorter and with distinet abdominal pattern 8

8 eyes at least moderately reduced (Figs. 66, 84) 9
- eyes well developed 10
9 eyes strongly reduced (Fig. 84) ..... *caverna*
- eyes moderately reduced (Fig. 66) ..... *blanda*
10 AME larger than PME (Figs. 163, 164, 212) 11
- AME equally sized or smaller than PME 12
11 CL shorter than 4 mm; male with distal sclerite of MA spoon-shaped, rounded (Figs. 208, 209); female with epigynal posterior sclerite dumbbell-shaped (Fig. 215) ..... *xilitla*
- CL longer than 4.5 mm; male with distal sclerite of MA long, subtriangular and pointed (Figs. 165, 167); female with epigynal posterior sclerite as in Fig. 170 ..... *rothi*
12 male pedipalp with elongated femur and tibia (Fig. 140); female with posterior membrane (internal posterior limit of genital area) distinctly protruding anteriad (Figs. 148, 149) ..... *mexicana*
- male palpal femur and tibia not elongated; female with posterior membrane only moderately protruding, sometimes with median notch 13
13 RTA with dorsal branch distally hook-shaped (Fig. 136); female with dumbbell-shaped posterior sclerite (posterior and anterior margins concave, Fig. 131) ..... *guanato*
- RTA with dorsal branch distally pointed or truncated; posterior epigynal sclerite with anterior margin concave only .. 14
14 MA with distal sclerite distally pointed (Figs. 95, 161); female with prominent or elongated appendages at genital duct (Figs. 99–101, 158) 15
- MA with distal sclerite spoon-shaped, distally rounded; female with subcircular, less prominent appendages at genital duct 16
15 MA with distal sclerite long triangular, sharply pointed (Figs. 159, 161); female vulva with arc-shaped anterior part (Fig. 158) ..... *queretaro*
- MA with distal sclerite subtriangular (Figs. 93, 95); female with distinctly elongated appendages at genital duct (Figs. 99–101) ..... *decora*
16 sternum with distinct pale patch only at the anterior half (Fig. 103); PLS with distal segment as long as basal segment (Fig. 104); sperm duct at tegulum without distinct curve (Fig. 111); female with semicircular posterior selerite (Fig. 105) ..... *fernandoi*
- sternum with different pattern; PLS with distal segment longer than basal segment (Fig. 118); sperm duct at tegulum with distinct curve (Fig. 195); female posterior sclerite with anterior margin concave, posterior margin straight 17
17 sternum with pale median line; MA with distal sclerite narrow, connection between tegulum and conductor narrow (Figs. 195–197); female as in Figs. 202–206 ..... *tlaxcala*
- sternum without pattern; MA with distal sclerite broad, connection between tegulum and conductor broad (Figs. 115, 116); female as in Figs. 122–125 ..... *gertschi*

Eratigena agrestis (Walckenaer, 1802)

Aranea agrestis Walckenaer, 1802: 216. Full synonymy: see Bolzern et al. (2013a).

Diagnosis.—Male *Eratigena agrestis* are similar to specimens of *E. atrica* in having a pocket-like (Roth 1968: "shell-like") median apophysis but differ from all other *Eratigena* species in having a massive and broad conductor with strongly sclerotized, pointed terminal appendages (Bolzern et al. 2013a: figs. 8 C–D, 9 A–B). Females differ in having a distinct epigyne with protruding posterior sclerite and deep anterior cavity (Bolzern et al. 2013a: fig. 9 D).

Distribution.—Introduced from Europe. In the Western Hemisphere, reported from several north-western states and New York (USA), and south-western parts of Canada.

Eratigena atrica (C. L. Koch, 1843)

Tegenaria atrica C. L. Koch, 1843: 105, fig. 825. Full synonymy: see Bolzern et al. (2013a).

Diagnosis.—*Eratigena atrica* specimens are similar to specimens of *E. agrestis* in having a pocket-like median apophysis, but differ from all other species in having the strongly sclerotized, finger-shaped and pointed dorsal branch of the RTA originating on a protuberance, and a strong conductor (Bolzern et al. 2013a: fig. 9 J–O). Females differ in having the large epigynal area as long as wide with distinct 'pseudo teeth', and having a uniquely shaped vulva (Bolzern et al. 2013a: fig. 10 A–F).

Distribution.—Introduced from Europe. In the Western Hemisphere, reported from the north-western United States and southern Canada (from east to west).

THE *FLEXUOSA*-GROUP

The *flexuosa*-group comprises four species: *E. edmundoi* sp. nov., *E. flexuosa* (F. O. Pickard-Cambridge, 1902) comb. nov., *E. florea* (Brignoli, 1974) comb. nov., and *E. yarini* sp. nov. The elongated distal part of the conductor (Figs. 27, 34, 52, 64) and the strongly elongated and coiled copulatory duct (anterior part, Figs. 19, 41, 45, 61) separate them from species of the *mexicana*-group.

Eratigena edmundoi sp. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:67CC827B-EF04-4C8F-AB60-6D7C7340C29A
Figs. 2, 3, 17–29

Type material.—*Holotype female*. MEXICO: *Veracruz*: Pico de Orizaba Volcano, Atotonilco de Calcahualco, plot 1, 2300

Figures 2–7.—Photographs of habitats and webs. 2 & 3, *Eratigena edmundoi* sp. nov. from Ajalpan, street between Pala and Nicolás Bravo, 2619 m (Mexico); 4 & 5, *Eratigena decora* Gertsch, 1971 from Xilitla, Ahuacatlán, ESE. of Potrerillos, Cueva de Potrerillos, 1181 m (Mexico); 6, *Eratigena queretaro* sp. nov. from Pinal de Amoles, at road 120 between Jalpan de Serra and Pinal de Amoles, close to La Curva del Chuveje, 20 km W. of Jalpan, 1288 m (Mexico); 7, *Eratigena caverna* Gertsch, 1971 from Peñamiller, Cueva Puerto del Léon, 6.5 km SE. of Río Blanco, 2484 m (Mexico).

m, 14 February 2012, Lab. Aracnologia, F. Alvarez Padilla (FC-UNAM: AB1340).

Paratypes. MEXICO: *Veracruz*: 1 ♂, same data as holotype (FC-UNAM: AB1340); 1 ♀, same data (FC-UNAM: AB1343: accession-nr. LN887151, LN887174); 1 ♂, same data (FC-UNAM: AB1338).

Other material examined.—MEXICO: *Puebla*: 1 ♀, 3 juv., Ajalpan, street between Pala and Nicolás Bravo, 2619 m, oak-pine forest, at terrain break at steep slope near path in the forest, 7 October 2014, A. Bolzern, E. González Santillán (NMB-ARAN-27500: AB1276); 1 ♂, 4 ♀, 2 juv., same data except 2545 m, pine forest, at terrain break (NMB-ARAN-27501 to 27502: AB1275, AB1255: accession-nr. LN887150, LN887173); 2 ♀, Zoquitlán, 2nd river cave, 31 December 1977, P. Strickland (AMNH: AB1148). *Veracruz*: 1 ♀, Volcán San Martin, near San Andres, 1524 m, 14 July 1953, C.J. Goodnight (AMNH: AB1168).

Etymology.—The specific name is a patronym in honor of Edmundo González Santillán, a Mexican arachnologist, expedition guide and friend.

Diagnosis.—Male *E. edmundoi* sp. nov. specimens differ from others of the group by the long triangular distal sclerite

at the median apophysis (Figs. 27, 28; rather than spoon shaped in *E. yarini* sp. nov. and *E. flexuosa*, moderately reduced in *E. florea*). They differ from *E. yarini* sp. nov. by the larger size (CL longer than 3.5 mm; in *E. yarini* sp. nov. shorter than 3 mm), and by the s-shaped subtegular sperm duct (Fig. 27; rather than c-shaped), and from *E. flexuosa* by the only moderately undulated tegular sperm duct (Fig. 27; rather than strongly undulated). Female specimens differ from all others of the group by the single coiled copulatory duct (Fig. 19; rather than only flattened in *E. yarini* sp. nov., double coiled in *E. flexuosa* and *E. florea*). In addition, specimens of *E. edmundoi* sp. nov. differ in having the distal segment of the PLS only proximally darkened (Fig. 17).

Description.—*Measurements:* Male (paratype): CL 4.0, CW 3.17, STL 1.97, STW 1.93, OL 4.27, OW 2.6. Leg I (6.8, 1.73, 6.4, 6.8, 3.3), II (5.87, 1.67, 3.67, 5.8, 2.93), III (5.27, 1.33, 4.07, 5.4, 2.4), IV (6.47, 1.6, 5.53, 7.4, 3.33), Pedipalp (1.83, 0.67, 1.0, 1.67), bulbL 1.0. Female (holotype): CL 4.01, CW 3.0, STL 1.8, STW 1.8, OL 5.33, OW 3.87. Leg I (5.2, 1.53, 4.66, 4.93, 2.6), II (4.27, 1.4, 3.53, 4.27, 2.0), III (3.93, 1.2, 3.2, 3.93, 1.87), IV (5.2, 1.4, 4.2, 5.4, 2.27). Pedipalp (1.77, 0.73, 1.23, 1.87). EPL 0.33, EPW 0.63. Eyes: eye rows moderately procurved (Fig. 23). PME 0.21, PLE 0.26, AME 0.24, ALE 0.23. Eye distances: PME–PME 1 x PME, PME–AME 0.5–0.75 x PME, PME–PLE 0.5 x PME, PME–ALE 0.75–1 x PME, AME–AME 0.5 x AME, AME–ALE 0.25 x AME. CLY1 1.5 x AME, CLY2 0.5 x ALE. *Male pedipalp:* RTA with two branches, lateral branch simple, lobe-like, moderately protruding, dorsal branch strongly sclerotized, narrow finger shaped, sharply pointed. Short dorsal spike on palpal tibia absent. Embolus length about 3.5 x CB, originating at 7–8 o'clock position, distal tip at 3–4 o'clock position. Conductor lamelliform, distal portion (DP) distinctly elongated, lateral margin folded (Figs. 27, 28). Terminal end of conductor strongly elongated, pointed. Transversal ridge (TR) of conduetor expressed as hyaline ridge (Fig. 27). Conductor membranously connected to tegulum. MA originating at 4–5 o'clock position, protruding, longer than wide, distal sclerite (DS) translucent, long triangularly shaped (Figs. 27, 28). MA membranously connected to tegulum. *Epigyne and vulva*: Epigyne medially with a pale, hyaline area (Fig. 18). Posterior sclerite protruding anteroventral as moderately sclerotized bar with anterior margin concave (semicircular, Fig. 18), posterior membrane (PM, internal posterior limitation of genital area) notched (Fig. 19). CO laterally of posterior sclerite. Epigynal 'pseudo teeth' present, small (Fig. 20). Vulva consists of combined narrowly convoluted duct, CD less sclerotized, with one coil, without appendages, RC stronger sclerotized (Fig. 19). FD only represented by small, leaf-shaped appendages (Fig. 22). *Other important characters*: Cheliceral promargin with 4–5, retromargin with 7–8 teeth, more proximally, the teeth become smaller. Colulus developed as trapezoidal plate with distal margin w-shaped. PMS bearing one conspicuously prominent spigot. PLS with distal segment nearly as long as basal segment (Fig. 25). Trichobothria on cymbium and palpal tarsus absent. Tarsal trichobothria at leg I 6–7. Small teeth on paired claws of leg I 10–11. Leg spination: male pedipalp (2-0-0 or 2-1-0, 2-0-0, 1-1+1p-0), female pedipalp (3-0-0, 2-0-0, 2-2-0), leg femora (1-3-2-0, 1-2-2-0 or 1-3-2-0, 1-2-2-0, 1-0-1-0 or 1-0-2-0 or 1-1-2-0), patellae (all 2-0-0), tibiae (0-0-0-4p or 0-1-0-1+3p, 0-0-0-1p+2+1p or 0-1-0-1p+2+1p, 1-2-2-2+2p or 2-2-2-2+2p, 2-2-2-3+1p), metatarsi (0-0-0-4p+1, 0-1-0-4p+1, 0-3-2-4p+1 or 0-3-3-4p+1, 0-3-3-3p+1+1p+1), tarsi (0, 0, 0-2-3-0, 0-2-3-0). *Coloration*: Carapace with two longitudinal symmetrical dark bands, irregularly expressed, margins narrowly darkened, head region dorsolaterally with two distinct, longitudinally curved dark bands (Fig. 17). Chelicerae frontally with distinct dark patches (Fig. 23). Sternum darkened anteriorly, sometimes also posteriorly (less distinct) with pale median band (Fig. 24). Opisthosoma dark, black, with reddish pale median band, anteriorly bordered by black bands or almost completely black, posteriorly with yellowish, reddish chevrons, indistinct (Fig. 17). Legs irregularly annulated. Colulus laterally darkened. ALS moderately, basal segment of PLS distinctly darkened, distal segment of PLS only proximal half darkened.

Distribution.—Reported from the two states Puebla and Veracruz (Mexico).

Eratigena flexuosa (F.O. Pickard-Cambridge, 1902), comb. nov.
Figs. 30–41

Tegenaria flexuosa F.O. Pickard-Cambridge, 1902: 334, pl. 31, fig. 34; Roth, 1968: 14, figs. 19, 20.

Type material.—*Holotype male*. MEXICO: *Guerrero*: Omiltemi ("Omilteme" on label), 28 April 1900, F.D. Godman (NHML).

Other material examined.—MEXICO: *Guerrero*: 1 ♀, Chilpancingo, Cueva del Borrego, 3.5 km E of Omiltemi, 2623 m, pine-oak forest, 23 July 2009, A. Valdez, O. Francke, H. Montaño, C. Santibáñez, T. Palafox, C. Trajano (CNAN: AB1199); 3 ♀, same data except Cueva del Borrego, 2 km E of Omiltemi, 1835 m, vegetation outside the cave, 20 June 2007, O. Francke, H. Montaño, L. Escalante, A. Ballesteros (CNAN: AB1202).

Diagnosis.—Male *E. flexuosa* specimens differ from others of the group by the strongly undulated tegular sperm duct (Fig. 34; rather than moderately undulated). They differ from *E. edmundoi* sp. nov. and *E. florea* by the spoon-like distal sclerite of the MA (Figs. 33, 34; rather than long triangular or moderately reduced). They differ from *E. yarini* sp. nov. by the larger size (CL longer than 3.5 mm; in *E. yarini* sp. nov. shorter than 3 mm), and by the s-shaped subtegular sperm duct (Fig. 34; rather than c-shaped). Female specimens differ from *E. edmundoi* sp. nov. and *E. yarini* sp. nov. by the double coiled copulatory duct (Fig. 41; rather than single coiled or only flattened), and from *E. florea* in having the epigynal median area bordered by a pronounced broad ridge (Fig. 38).

Description.—Essential information for the male was provided by F.O. Pickard-Cambridge (1902). *Measurements:* Female (AB1199): CL 3.75, CW 2.67, STL 2.0, STW 1.63, OL 3.67, OW 2.33. Leg I (6.13, 1.60, 5.67, 6.00, 2.8), II (5.00, 1.47, 4.47, 5.13, 2.33), III (4.40, 1.33, 3.60, 4.87, 2.20), IV (5.27, 1.47, 5.0, 6.87, 2.40). Pedipalp (1.97, 0.67, 1.17, 2.0). EPL 0.56, EPW 0.88. Eyes: anterior eye row straight, posterior eye row procurved (Fig. 37). PME 0.17, PLE 0.19 , AME 0.15, ALE 0.22. Eye distances: PME–PME 0.75 x PME, PME–AME 1 x PME, PME–PLE 1 x PME, PME–ALE 1.5 x PME, AME–AME 0.5 x AME, AME–ALE 0.5 x AME. CLY1 1.5 x AME,

CLY2 1.25 x ALE. *Epigyne and vulva*: Epigyne with hyaline area subrectangular, bordered by pronounced broad ridge (Fig. 38). Posterior membrane without notch (Fig. 41). CO anterolateral to posterior sclerite. Epigynal 'pseudo teeth' indistinct (Fig. 39). Vulva consists of combined narrowly convoluted duct, CD less sclerotized, almost double coiled, without appendages, RC more strongly sclerotized (Fig 41). FD only represented by small, leaf-shaped appendages. *Other important characters*: Cheliceral promargin with 4, retromargin with 6 teeth, more proximally, the teeth become smaller (Fig. 36). Colulus developed as trapezoidal plate with distal margin moderately w-shaped. PMS bearing one conspicuously prominent spigot. PLS with distal segment moderately longer than basal segment (Fig. 32). Trichobothria on cymbium and palpal tarsus absent. Tarsal trichobothria of leg I 7. Small teeth on paired claws of leg I 10–11. Leg spination: female pedipalp (1-0-0 or 2-0-0, 2-0-0, 2-1-2), leg femora (1-3-1-0 or 1-3-2-0, 1-2-2-0 or 1-3-2-0, 1-0-2-0 or 1-1-2-0, 1-0-1-0), patellae (all 2-0-0), tibiae (0-0-0-1 or 0-1-0-1, 0-1-0-1p or 1-1-0-1p, 2-1-1-2p, 2-1-1-1), metatarsi (0-0-0-1p+1+1p+1, 0-0-0-1p+2+1p+1, 0-1-1-4p+1 or 0-1-2-4p+1, 0-2-1-4p+1 or 0-2-2-4p+1), tarsi (0, 0, 0-0-1-0, 0-0-2-0). *Coloration*: Carapace with two longitudinal symmetrical dark bands, irregularly and indistinctly expressed, margins narrowly darkened, head region dorsolateral with two indistinct, longitudinally curved dark bands (Fig. 30). Chelicerae frontally without dark patches (Fig. 37). Sternum darkened, with indistinct pale median band (Fig. 31). Opisthosoma dark brownish to black, with yellowish pale median band, anteriorly with two longitudinally dark bands, posteriorly with yellowish chevrons (Fig. 30). Legs irregularly annulated. Colulus pale or laterally moderately darkened. ALS and both segments of PLS moderately darkened.

Distribution.—Reported only from the state of Guerrero (Mexico).

Comments.—Based on the drawing provided by F.O. Pickard-Cambridge (1902), Roth stated that the median apophysis is lacking (Roth 1968: 14), but this was a misinterpretation (see Figs. 33–35). Males and females have never been collected together (the only known male is the holotype). However, based on their morphological similarity and their collection close to the type locality, these females are tentatively placed with this species.

Eratigena florea (Brignoli, 1974), comb. nov.
Figs.42–53

Tegenaria florea Brignoli, 1974: 228, fig. 10A, C.
Tegenaria prope *florea*: Brignoli, 1974: 231, fig. 10B.

Type material.—*Holotype female*. MEXICO: *Chiapas*: Comitán, Cueva de las Florecillas, 2265 m, 18 March 1971, A. Zollini (MCSNV).

Other material examined.—MEXICO: *Chiapas*: 1 ♀, Amatenango, Cueva I de Tulanca, 2200 m, 4 March 1971, V. Sbordoni (MCSNV); 1 ♀, Comitán, Cueva de la Cruz Belen, 2210 m, 19 March 1971, R. Argano (MCSNV, sub prope *florea*); 1 ♀, 5 miles West of San Cristobal, 24 August 1966, W. & J. Ivie (AMNH: AB1112); 1 ♀, same data except 2438 m, pine-oak forest, 16 August to 3 September 1969, S. & J. Peck (AMNH: AB1166); 1 ♂, Laguna Bélgica Educational Park, 16 km NW. of Ocozocoautla de Espinosa, 14 June 1990, H. Howden (AMNH: AB1141). *Oaxaca*: 1 ♀, Santa María Tlahuitoltepec, Distrito Mixes, 2032 m, pine forest, disturbed, 14 September 2009, A. Valdez, C. Santibáñez, R. Paredes (CNAN: AB1193); 1 ♀, Yautepec, Santo Tomás Teipan, 2346 m, cloud forest, 20 March 2002, S. Reynaud (CNAN: AB1200).

Diagnosis.—Male *E. florea* specimens differ from others of the group by the moderately reduced distal sclerite of the median apophysis (Figs. 51, 52; rather than long and triangular in *E. ednundoi* sp. nov., spoon shaped in *E. yarini* sp. nov. and *E. flexuosa*). Female specimens differ from *E. ednundoi* sp. nov. and *E. yarini* sp. nov. by the double coiled copulatory duct (Fig. 43; rather than single coiled or only flattened), and from *E. flexuosa* in having the epigynal median area not bordered by a pronounced broad ridge (Fig. 42).

Description.—Essential information for the female was provided by Brignoli (1974). *Measurements:* Male (AB1141): CL 2.9, CW 2.17, STL 1.42, STW 1.34, OL 3.17, OW 2.1. Leg I (5.25, 1.15, 5.15, 5.8, 3.05), II (4.6, 1.0, 3.98, 4.5, 2.1), III (4.1, 1.0, 3.65, 4.5, 2.25), IV (5.35, 1.0, 4.75, 6.4, 2.8), Pedipalp (1.3, 0.44, 0.56, 1.18), bulbL 0.84. Eyes: eye rows moderately procurved (Fig. 48). PME 0.17, PLE 0.18 , AME 0.16, ALE 0.22. Eye distances: PME–PME 0.5 x PME, PME–AME 0.5 x PME, PME–PLE 0.4 x PME, PME–ALE 0.7 x PME, AME–AME 0.5 x AME, AME–ALE 0.25 x AME. CLY1 1.5–2 x AME, CLY2 0.75–1 x ALE. *Male pedipalp:* RTA with two branches, lateral branch simple, lobe-like, moderately protruding, dorsal branch strongly sclerotized, narrow finger shaped, sharply pointed. Short dorsal spike on palpal tibia absent. Embolus length about 3.5 x CB, originating at 7–8 o'clock position, distal tip at 4 o'elock position. Conductor lamelliform, distal portion distinctly elongated, lateral margin folded (Figs. 51–53). Terminal end of conductor strongly elongated, pointed. Transversal ridge of conductor expressed as hyaline ridge. Conductor membranously connected to tegulum. MA originating at 4–5 o'cloek position, as long as wide, distal sclerite translucent, plate like, moderately reduced (Figs. 51–53). *Other important characters*: Cheliceral promargin with 4–5, retromargin with 7–8 teeth, more proximally, the teeth become smaller. Colulus developed as rectangular to trapezoidal plate with distal margin w-shaped. PLS with distal segment slightly shorter than basal segment (Fig. 50). Trichobothria on cymbium and palpal tarsus absent. Tarsal trichobothria at leg I 6–7. Small teeth on paired claws of leg I 8. Leg spination: male pedipalp (2-0-0, 2-0-0, 1-1-0), leg femora (1-2-0-0, 1-0-0-0 or 1-1-0-0, 1-0-0-0, 1-0-0-0), patellae (all 2-0-0), tibiae (0-0-0-1, 0-0-0-1 or 1-0-0-1, 2-1-0-1, 2-1-1-1), metatarsi (0-0-0-1p+1+1p+1, 0-0-0-1p+1+1p+1 or 0-0-0-1p+2+1p+1, 0-2-1-1p+1+2p+1, 0-2-1-1p+2+1p+1), tarsi (0, 0, 0, 0-0-1-0). *Coloration*: Carapace with two longitudinal symmetrical dark bands, irregularly expressed, margins narrowly darkened (Fig. 47). Chelicerae frontally with indistinct dark patches (Fig. 48). Sternum indistinctly darkened, anteriorly with pale median band (Fig. 49). Opisthosoma dark, grayish brown, without reddish pigments, pale median band, posteriorly with pale, yellowish chevrons, indistinct (Fig. 47). Legs irregularly annulated, indistinct. Colulus moderately darkened. ALS and both segments of PLS darkened.

Distribution.—Reported from several localities in the states of Chiapas and Oaxaca (Mexico).

Comments.—Males and females have never been collected together. However, based on their morphological similarity and the collection site being within the distribution range of the females, the male from Laguna Bélgica Educational Park, 16 km NW. of Ocozocoautla de Espinosa, is tentatively placed in this species.

Eratigena yarini sp. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:FAFC2F99-20AC-439C-B177-E767115CA13B
Figs. 54–65

Type material.—*Holotype female*. MEXICO: *Veracruz*: Pico de Orizaba Volcano, Atotonilco de Calcahualco, plot 1, 2300 m, 24 February 2013, Lab. Araenologia, F. Alvarez Padilla (FC-UNAM: AB1342).

Paratypes. MEXICO: *Veracruz*: 1 ♂, same data as holotype (FC-UNAM: AB1341); 1 ♂, 1 ♀, same data (NMB-ARAN-27503 to 27504: AB1332, AB1334: accession-nr. LN887162, LN887181).

Other material examined.—MEXICO: *Oaxaca*: 1 ♀, "El Cumbre" on ridge E. of Cerro San Felipe, 2438 m, 28 September 1961, C.M. & M.R. Bogert (AMNH: AB1124).

Etymology.—The specific name is a patronym dedicated to Yarin Bolzern, the second born child of the first author.

Diagnosis.—*E. yarini* sp. nov. specimens differ from others of the group by the smaller size (CL shorter than 3.0 mm; all others longer than 3.5 mm). Males differ by the c-shaped subtegular sperm duct (arrow in Fig. 64; rather than s-shaped), females by the only flattened copulatory duct (Figs. 59–61; rather than single or double coiled).

Description.—*Measurements:* Male (paratype): CL 1.9, CW 1.5, STL 1.97, STW 1.93, OL 2.04, OW 1.26. Leg I (2.5, 1.0, 2.23, 2.02, 1.42), II (2.04, 0.68, 1.64, 1.78, 1.26), III (1.9, 0.62, 1.34, 1.8, 1.1), IV (2.67, 0.733, 2.2, 2.67, 1.4), Pedipalp (0.78, 0.32, 0.44, 0.68), bulbL 0.4. Female (holotype): CL 2.53, CW 1.83, STL 1.12, STW 1.14, OL 3.0, OW 2.12. Leg I (2.67, 0.87, 2.4, 2.4, 1.67), II (2.4, 0.7, 1.68, 1.88, 1.1), III (2.1, 0.68, 1.54, 1.9, 1.2), IV (2.87, 0.8, 2.28, 3.03, 1.3). Pedipalp (0.96, 0.42, 0.64, 1.08). EPL 0.38, EPW 0.58. Eyes: eye rows moderately procurved (Fig. 55). PME 0.1, PLE 0.12 , AME 0.08, ALE 0.1. Eye distances: PME–PME 0.5–1 x PME, PME–AME 0.5–1 x PME, PME–PLE 0.5–1 x PME, PME–ALE 1 x PME, AME–AME <0.5 x AME, AME–ALE <0.5 x AME. CLY1 2 x AME, CLY2 0.5–1 x ALE. *Male pedipalp:* RTA with two branches, lateral branch triangular, protruding, dorsal branch strongly sclerotized, narrow finger shaped, sharply pointed (Fig. 65). Short dorsal spike on palpal tibia absent. Embolus length about 2 x CB, originating at 8 o'clock position, distal tip at 4 o'clock position. Conductor lamelliform, distal portion distinctly elongated, lateral margin folded (Figs. 63–65). Terminal end of conductor strongly elongated, pointed. Transversal ridge of conductor expressed as hyaline ridge. Conductor membranously connected to tegulum. MA originating at 6 o'clock position, protruding, longer than wide, distal sclerite translucent, spoon-like (Figs. 63, 64). MA membranously connected to tegulum. *Epigyne and vulva*: Epigyne medially with a pale, hyaline area (Fig. 58). Posterior sclerite with anterior margin strongly coneave, posterior membrane broadly notched (Fig. 61). CO anterolateral to posterior sclerite. Epigynal 'pseudo teeth' present, small (Fig. 62). Vulva consists of combined narrowly convoluted duct, CD flattened, medially elongated, without appendages, RC stronger sclerotized (Figs. 59–61). FD only represented by small, leaf-shaped appendages. *Other important characters*: Cheliceral promargin with 4, retromargin with 5 (male) or 6 (female) teeth, most proximal tooth smaller. Colulus developed as trapezoidal plate with distal margin w-shaped. PMS bearing one conspicuously prominent spigot. PLS with distal segment nearly as long as basal segment (Fig. 57). Trichobothria on cymbium and palpal tarsus absent. Tarsal trichobothria at leg I 5–6. Small teeth on paired claws of leg I 7. Leg spination: male pedipalp (2-0-0, 2-0-0, 1-2-0), female pedipalp (2-0-0, 2-0-0, 2-2-0), leg femora (2-2-0-0, 2-0-0-0, 2-0-1-0, 1-0-1-0), patellae (all 2-0-0), tibiae (2-0-0-1+1p,2-1-0-2p, 2-2-1-3, 2-2-2-3), metatarsi (0-0-0-3p+1, 0-1-0-3p+1, 0-3-2-1p+1+2p+1, 0-3-2-1p+1+2p+1), tarsi (0, 0, 0-1-2-0, 0-2-3-0). *Coloration*: Carapace and head region with two broad longitudinal symmetrical dark bands, with triangular darker spots (Fig. 54). Chelicerae frontally with indistinct dark patches (Fig. 55). Sternum darkened, distinct pale median band, laterally with indistinet paler spots (Fig. 56). Opisthosoma dark, brownish, sprinkled with small pale spots, indistinct pale median band, posteriorly with yellowish chevrons, indistinct (Fig. 54). Leg femora broad but moderately annulated, other segments moderately annulated, tarsi pale. Colulus, ALS and PLS distinctly darkened (Fig. 57).

Distribution.—Reported only from the state of Veracruz (the type locality) and one locality in the state of Oaxaca (Mexico).

THE *MEXICANA*-GROUP

The *mexicana*-group comprises 12 speeies: *E. blanda* (Gertsch, 1971) comb. nov., *E. caverna* (Gertsch, 1971) comb. nov., *E. decora* (Gertsch, 1971) comb. nov., *E. fernandoi* sp. nov., *E. gertschi* (Roth, 1968) comb. nov., *E. guanato* sp. nov., *E. mexicana* (Roth, 1968) comb. nov., *E. queretaro* sp. nov., *E. rothi* (Gertsch, 1971) comb., nov., *E. selva* (Roth, 1968) comb. nov., *E. tlaxcala* (Roth, 1968) comb. nov., and *E. xilitla* sp. nov. The only moderately elongated distal portion of the conductor (Figs. 79, 82, 94, 112, 136, 142, 160, 166, 175, 187, 196, 208) and the short, straight copulatory duct with mostly distinct appendages (Figs. 73, 87, 101, 124, 133, 147, 149, 158, 185, 192, 206, 218) separate them from specimens of the *flexuosa*-group.

Eratigena blanda (Gertsch, 1971), comb. nov.
Figs. 66–71

Tegenaria blanda Gertsch, 1971: 105.

Type material.—*Holotype female*. MEXICO: *Tamaulipas*: El Porvenir, Cueva de la Capilla, 13.5 km NW Gómez Farías, 28 January 1969, J. Reddell, R. Mitchell, F. Rose, J. George (AMNH).

Other material examined.—MEXICO: *Tamaulipas*: 1 ♀, Gómez Farías, Cueva de la Perra, 15 mi. NW Gómez Farías, 2164 m, 28 January 1968, J. Reddell, R. Mitchell, F. Rose, J. George (AMNH: AB1153).

Diagnosis.—Females of *E. blanda* differ from all other Nearctic *Eratigena* species in having moderately reduced eyes (Fig. 66; rather than strongly redueed eyes in *E. caverna*, eyes normally developed in all other species).

Description.—Essential information was provided by Gertsch (1971).

Distribution.—Reported from two caves in the state of Tamaulipas (Mexico).

Eratigena caverna (Gertsch, 1971), comb. nov.
Figs. 7, 81–89

Tegenaria caverna Gertseh, 1971: 106, figs. 158–160.

Type material.—*Holotype male*. MEXICO: *Queretaro*: Peñamiller, Cueva Puerto del Léon, 6.5 km SE Río Blanco, 9 July 1967, J. Reddell, J. Fish, P. Russell (AMNH: AB1156).

Paratypes. MEXICO: *Queretaro*: 2 ♀ allotypes, same data as holotype (AMNH: AB1156).

Other material examined.—MEXICO: *Queretaro*: 5 juv., same data as holotype except deep inside cave, 2484 m, 14 October 2014, A. Bolzern, E. González Santillán (NMB-ARAN-27505 to 27507: AB1244, AB1305, AB1284: accession-nr. LN887148, LN887187).

Diagnosis.—Male and female *E. caverna* specimens differ from all other West Nearctic *Eratigena* species by having strongly reduced eyes (Fig. 84; rather than moderately reduced in *E. blanda*, eyes normally developed in all other species).

Description.—Essential information was provided by Gertsch (1971).

Distribution.—Reported only from a cave near Río Blanco in the state of Queretaro (Mexico).

Comments.—Specimens could not be detected at the entrance of the cave but only very deep inside the cave. There, they were quite abundant, building their webs (Fig. 7) between large rocks which covered the lower part of the cave. Interestingly, there was no tube- or funnel-shaped retreat deteetable in their webs. The spiders were just sitting in the middle of their web on a circular, more densely woven patch.

Eratigena decora (Gertsch, 1971), comb. nov.
Figs. 4, 5, 90–101

Tegenaria decora Gertsch, 1971: 104, figs. 164, 165.

Type material.—*Holotype male*. MEXICO: *San Luis Potosí*: Cueva de Potrerillos, 1.5 km W. of Ahuacatlán, 12 July 1967, J. Reddell, J. Fish, P. Russell (AMNH).

Paratypes. MEXICO: *San Luis Potosí*: 7 ♀, same data as holotype (AMNH: AB1167).

Other material examined.—MEXICO: *San Luis Potosí*: 1 ♂, 1 ♀, Xilitla, Ahuacatlán, ESE. of Potrerillos, Cueva de Potrerillos, 1181 m, cave entrance with tropical forest in the middle of pasture land, at rock faces and woody vegetation, 12 October 2014, A. Bolzern, E. González Santillán (NMB-ARAN-27508: AB1237: accession-nr. LN887149); 2 ♂, 5 ♀, 3 juv., same data (FC-UNAM: AB1240, AB1280).

Diagnosis.—Female *E. decora* specimen differ from all others of the group in having elongated appendages at the genital duct (Figs. 99–101). Males are similar to *E. guanato* sp. nov., *E. queretaro* sp. nov., *E. rothi* and *E. selva* in having at least a moderately pointed distal sclerite of the MA (Figs. 93, 95). They differ from *E. guanato* sp. nov., *E. rothi* and *E. selva* in having a relatively slim, finger-shaped and simply pointed dorsal branch of the RTA (rather than distally hook-shaped in *E. quanato* sp., nov. strong and basally bent in *E. rothi* and *E. selva*), and from *E. queretaro* sp. nov. in having a subtriangular, moderately pointed distal sclerite of the MA (rather than triangular and sharply pointed).

Description.—Essential information was provided by Gertsch (1971).

Distribution.—Reported only from one cave in the state of San Luis Potosí (Mexico).

Comments.—In 2014, specimens of *E. decora* were collected at the entrance of Cueva de Potrerillos (Figs. 4, 5), the type locality. Interestingly, this cave entrance, a large woody hole, is located in the middle of pasture land, surrounded by cattle and secured by a fence. It is remarkable that the webs of this species were also attached to the woody vegetation, and not exclusively to rocks (Fig. 5).

Eratigena fernandoi sp. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:19066CF1-0625-4D40-8FB7-35E0CF74AD8B
Figs. 102–113

Type material.—*Holotype male*. MEXICO: *Veracruz*: Atotonilco de Calcahualco, Pico de Orizaba Volcano, plot 2, 24 February 2013, Lab. Aracnologia FC-UNAM (FC-UNAM: AB1335: accession-nr. LN887152, LN887175).

Paratypes. MEXICO: *Veracruz*: 1 ♀, same data as holotype except 14 February 2012 (FC-UNAM: AB1333).

Other material examined.—MEXICO: *Veracruz*: 3 ♂, Huatusco, 7 km E Huatusco, cloud forest, 22 Jun 1983, S. & J. Peck (AMNH: AB1137).

Etymology.—The specific name is a patronym in honor of Fernando Alvarez Padilla, a Mexican arachnologist and active and strong supporter of the current work.

Diagnosis.—Male and female of *E. fernandoi* sp. nov. differ from related species in having a distinct pale patch only at the anterior half of the sternum (Fig. 103). Males are most similar to *E. gertschi* in having the posterior sclerite of the MA broadly spoon-shaped and distally rounded (rather than distally pointed in *E. decora*, *E. guanato* sp. nov., *E. rothi* and *E. selva*), but differ from that species in having the distal segment of the PLS as long as the basal segment (Fig. 104; rather than distal segment longer than basal segment), and the spermatic duct of the tegulum without a distinct curve (arrow in Fig. 111; rather than distinctly curved). Females differ by the semicircular posterior sclerite (Fig. 105), and the anteriorly differently convoluted genital ducts (Fig. 106).

Description.—*Measurements:* Male (holotype): CL 2.26, CW 1.7, STL 1.06, STW 1.14, OL 3.0, OW 1.74. Leg I (4.0, 0.93, 3.8, 3.8, 2.33), II (3.57, 0.9, 2.97, 3.6, 2.03), III (3.13, 0.8, 2.63, 3.43, 1.7), IV (3.93, 0.93, 3.43, 4.35, 2.1), Pedipalp (1.2, 0.42, 0.68, 1.03), bulbL 0.44. Female (paratype): CL 3.2, CW 2.4, STL 1.8, STW 1.8, OL 3.3, OW 2.47. Leg I (4.25, 1.1, 3.75, 3.95, 2.3), II (3.87, 1.1, 3.23, 3.6, 1.76), III (3.3, 1.0, 2.73, 3.47, 1.6), IV (4.25, 1.0, 3.7, 4.65, 1.92). Pedipalp (1.36, 0.56, 0.94, 1.11). EPL 0.33, EPW 0.54. Eyes: anterior eye row straight, posterior moderately procurved (Fig. 102). PME 0.13, PLE 0.14 , AME 0.12, ALE 0.17. Eye distances: PME–PME 1 x PME, PME–AME 0.5–0.75 x PME, PME–PLE 1 x PME,

PME–ALE 1.25 x PME, AME–AME 0.5–0.75 x AME, AME–ALE 0.5 x AME. CLY1 2 x AME, CLY2 1.5 x ALE. *Male pedipalp:* RTA with two branches, lateral branch simple, lobe-like, moderately protruding, dorsal branch strongly sclerotized, narrow finger shaped, sharply pointed (broken off in Figs. 112, 113). Short dorsal spike on palpal tibia absent. Embolus length about 1.5 x CB, originating at 9–10 o'clock position, distal tip at 4 o'clock position. Conductor lamelliform, distal portion only moderately elongated, lateral margin folded (Figs. 112, 113). Terminal end of conductor strongly elongated, pointed. Transversal ridge of conductor expressed as hyaline ridge (Figs. 111, 112). Conductor membranously connected to tegulum. MA originating at 5 o'clock position, protruding, longer than wide, distal sclerite translucent, broad spoon shaped. MA membranously connected to tegulum. *Epigyne and vulva*: Epigyne medially with a pale, hyaline area, oval (Fig. 105). Posterior sclerite protruding anteroventral as moderately sclerotized bar with anterior margin concave (semicircular, Fig. 105), posterior membrane notched (Fig. 106). CO anterolateral to posterior sclerite. Epigynal 'pseudo teeth' present, small. Vulva consists of combined narrowly convoluted duct, CD less selerotized, with indistinct appendages, RC stronger sclerotized (Figs. 106, 107). FD only represented by small, leaf-shaped appendages. *Other important characters*: Cheliceral promargin with 4, retromargin with 6 small teeth in two separated groups of 3, most proximal tooth smaller. Colulus developed as trapezoidal plate with distal margin moderately w-shaped (Fig. 104). PMS bearing one conspicuously prominent spigot. PLS with distal segment nearly as long as basal segment (Fig. 104). Trichobothria on cymbium and palpal tarsus absent. Tarsal trichobothria at leg I 6. Small teeth on paired claws of leg I 7–10. Leg spination: male pedipalp (2-0-0, 2-0-0, 1-2-0), female pedipalp (3-0-0, 2-0-0, 2-2-0), leg femora (1-2-0-0 or 1-3-1-0, 1-2-2-0 or 1-1-2-0, 1-0-2-0 or 1-2-2-0, 1-0-2-0), patellae (all 2-0-0), tibiae (0-1-0-1 or 1-1-0-1, 0-1-0-1 or 1-1-0-1, 2-1-1-1 or 2-2-1-2, 2-2-2-3), metatarsi (0-0-0-4p+1, 0-0-0-4p+1 or 0-1-0-4p+1, 0-2-2-4p+1 or 0-3-3-4p+1, 0-3-3-4p+1), tarsi (0, 0, 0-0-1-0, 0-1-3-0 or 0-2-2-0). *Coloration*: Carapace with two longitudinal symmetrical dark bands, irregularly expressed, margins narrowly darkened, head region dorsolaterally with two distinct, longitudinally curved dark bands (Fig. 109). Chelicerae frontally with distinct dark patches (Fig. 102). Sternum darkened, anterior half with pale median band (Fig. 103). Opisthosoma greenish to dark gray, without reddish pigments, indistinct yellowish median band, anteriorly bordered by dark bands, posteriorly with yellowish chevrons, indistinct (Fig. 109). Legs irregularly but distinctly annulated. Colulus completely darkened. ALS moderately, basal segment of PLS distinctly darkened, distal segment of PLS only proximal half darkened.

Distribution.—Reported only from two localities in the state of Veracruz (Mexico).

Eratigena gertschi (Roth, 1968), comb. nov.
Figs. 114–125, cf. *gertschi* Figs. 72–80

Tegenaria mexicana gertschi Roth, 1968: 22, fig. 27.
Tegenaria gertschi Roth: Brignoli, 1974: 230.

Type material.—*Holotype male*. MEXICO: *Nuevo León*: Resumidero (cave) de Pablillo at Hacienda Pablillo, 30 km south of Galeana, 4 June 1966, J. Reddell, D. McKenzie (AMNH).

Other material examined (of *E. gertschi* s. s.).—MEXICO: *Nuevo León*: 1 ♀, Monterrey, Chipinque Mesa, small caves, 1645 m, 24 June 1969, S. & J. Peck, R. Norten (AMNH: AB1106). *Tamaulipas*: 2 ♀, Gómez Farías, 6 miles NW. of Gómez Farías, mine cave, March 1969, J. Reddell, C. Tucker (AMNH: AB1161); 1 ♀, Rancho del Cielo, mine cave, 3 June 1967, R. Mitchell (AMNH: AB1135); 1 ♀, same data except 10 January 1971, J. Reddell (AMNH: AB1163).

Other material examined (of *E.* cf. *gertschi*).—MEXICO: *Tamaulipas*: 1 ♀, Ejido Conrado Castillo, Cerro Zapatero, Cueva del Coral, 19 March 1979, D. Pate, J. Atkinson, M. Shumate (AMNH, AB1102); 1 ♂, San Juan, La Cueva sin Nombra, 4 June 1967, R. Mitchell (AMNH: AB1165); 1 ♂, Sótano de Monumento, 26 km WNW of Ocampo, near Allende, 5 September 1979, W.R. Elliott, D.C. Rudolph (AMNH: AB1142).

Diagnosis.—Male and female of *E. gertschi* are similar to *E. mexicana* in having the distal segment of the PLS twice as long as the basal segment (Fig. 118; *E. xilitla* sp. nov. with distal to basal segment 3/2). Males differ from *E. mexicana* by the normal proportions of the palpal femur and tibia (Fig. 117; rather than both strongly elongated), females in having an only moderately elevated and medially notched posterior membrane (Fig. 125; rather than strongly elevated). Males of *E. gertschi* are similar to *E. fernandoi* sp. nov. in having the posterior sclerite of the MA broadly spoon-shaped and distally rounded (Fig. 116; rather than distally pointed in *E. decora*, *E. guanato* sp. nov., *E. rothi* and *E. selva*), but differ from this species in having the spermatic duct of the tegulum distinctly curved (arrow in Fig. 114; rather than without distinct curve). Females are similar to specimens of *E. guanato* sp. nov., *E. rothi, E. tlaxcala,* and *E. xilitla* sp. nov. but differ from *E. guanato* sp. nov. in having the posterior sclerite not dumbbell-shaped (Fig. 122), from *E. rothi* and *E. xilitla* sp. nov. in having the AME only as large as the PME, and from *E. tlaxcala* by the terminal part of the vulva (Figs. 123, 124).

Description.—Essential information for the male was provided by Roth (1968: 22–23). *Measurements:* Female (AB1106): CL 4.1, CW 3.1, STL 1.9, STW 1.72, OL 5.25, OW 3.6. Leg I (6.45, 1.7, 6.1, 6.2, 2.65), II (5.6, 1.45, 4.55, 5.2, 2.25), III (5.05, 1.25, 3.95, 5.2, 2.05), IV (6.5, 1.57, 5.55, 7.25, 2.5). Pedipalp (2.17, 0.8, 1.33, 1.97). EPL 0.43, EPW 0.81. Eyes: anterior eye row straight, posterior eye row moderately procurved. PME 0.18, PLE 0.23, AME 0.14, ALE 0.21. Eye distances: PME–PME 1.5 x PME, PME–AME 1.25 x PME, PME–PLE 1.25 x PME, PME–ALE 1.5 x PME, AME–AME 0.75–1 x AME, AME–ALE 0.8 x AME. CLY1 2.5 x AME, CLY2 1.3 x ALE. *Epigyne and vulva*: Epigyne medially with a pale, hyaline area (Fig. 122). Posterior sclerite protruding anteroventral as moderately sclerotized bar with anterior margin concave (Figs. 122, 125), posterior membrane notched (Fig. 125). CO laterally of posterior sclerite. Epigynal 'pseudo teeth' present, small (Fig. 122). Vulva consists of combined narrowly convoluted duct, CD less sclerotized, with appendages (Figs. 124, 125), RC stronger sclerotized, terminally strongly convoluted (Figs. 123, 124). FD only represented by small, leaf-shaped appendages. *Other important characters*: Chelieeral promargin with 4, retromargin with 6 teeth, more

proximally, the teeth become smaller. Colulus developed as trapezoidal plate with distal margin w-shaped. PMS bearing one conspicuously prominent spigot. PLS with distal segment twice as long as basal segment (Fig. 118). Trichobothria on cymbium and palpal tarsus absent. Tarsal trichobothria at leg I 7. Small teeth on paired claws of leg I 10–11. Leg spination: female pedipalp (0-0-0 or 1-0-0, 2-0-0, 2-1+1p-0), leg femora (1-2-0-0, 1-1-1-0, 1-0-0-0, 1-0-0-0), patellae (all 2-0-0), tibiae (0-0-0-0, 0-0-0-0, 0-0-0-0 or 2-0-0-0, 2-0-0-1), metatarsi (0-0-0-4p+1, 0-1-0-4p+1, 0-2-3-4p+1, 0-2-3-4p+1), tarsi (0, 0, 0, 0-0-1-0). *Coloration*: Carapace with two longitudinal symmetrical dark bands, irregularly expressed, margins narrowly darkened, head region darker, laterally with indistinct dark patches (Fig. 121). Chelicerae frontally without dark patches. Sternum darkened, medially moderately paler (Fig. 119). Opisthosoma dark grayish, without red pigments, anteriorly with dark patch, bordered by yellowish bands, posteriorly with yellowish chevrons (Fig. 120). Legs very indistinctly annulated. Colulus pale. ALS pale, basal segment of PLS darkened, distal segment of PLS only proximally darkened (2/3).

Distribution.—Reported from localities in the states of Nuevo León and Tamaulipas (Mexico).

Comments.—One female and two males from different localities in Tamaulipas differ from *E. gertschi* s. s. by the different vulva with an apparently fused duct (Figs. 73, 74), and the narrow and pointed distal sclerite of the MA (Figs. 78–80). Due to the fact that: (i) the species group in focus comprises a complex of very closely related species, (ii) the males and female were not collected at the same locality and (iii) only one female is available, these morphs are not described as a new species and treated here as *E.* cf. *gertschi*.

Eratigena guanato sp. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:9AC79754-8337-4AC5-97A2-387C757A78C2
Figs. 126–137

Type material.—*Holotype female*. MEXICO: *Guanajuato*: Guanajuato, at road 110 from Dolores Hidalgo to Guanajuato, 1 km N of Santa Rosa de Lima, 2510 m, oak forest, 16 October 2014, A. Bolzern, E. González Santillán (FC-UNAM: AB1260).

Paratypes. MEXICO: *Guanajuato*: 5 juv., same data as holotype (FC-UNAM: AB1260); 3 ♀, 3 juv., same data (NMB-ARAN-27509: AB1243: accession-nr. LN887176).

Other material examined (of *E. guanato* s. s.).—MEXICO: *Unknown:* 1 ♀, 280 Cueva las Calevas, 6 April 1941, Mich (AMNH: AB1130). *Colima*: 1 ♀, Nevado de Colima, 20 January 1943, F. Bonet (AMNH: AB1126). *Jalico:* 1 ♀, Mascota, km 21at street from Maseota to Puerto Vallarta, 1662 m, pine forest, night catch, 1 April 2012, L. Olguin, J. Mendoza, G. Contreraz, C. Santibañez, D. Ortiz (CNAN). *Michoacán:* 1 ♂, Basencheve National Park, 7 May 1963, W.J. Gertsch, W. Ivie (AMNH: AB1103); 1 ♀, Garnica Pass, 2834 m, 8 May 1963, W.J. Gertsch, W. Ivie (AMNH: AB1127); 1 ♀, Cd. Hidalgo, Gruta de Tziranda, 1855 m, 29 April 2011, A. Valdez, O. Franeke, J.A. Cruz, R. Monjaraz, E. Miranda (CNAN); 1 ♀, Zitacuaro, ca. 30 km SE of Zitacuaro, Butterfly forest, 2896 m, 16 December 2011, S. Huber (NMB-ARAN-27510: AB1089).

Other material examined (of *E.* cf. *guanato*).—MEXICO: *Hidalgo*: 2 ♀, 5 miles SW. of Jacala, 21 April 1963, W.J. Gertsch, W. Ivie (AMNH: 1176). *Michoacán*: 1 ♀, Coalcomán, Cueva de Cascada Chica, 10 km NE Coalcomán de Matamoros, 1356 m, 1 May 1984, L. Elliott, D. McKenzie (AMNH: AB1100); 1 ♀, 15 km NE. of Coalcomán de Matamoros, Cueva de Torrecillas, 2 May 1984, D. McKenzie, L. Elliot (AMNH: AB1108).

Etymology.—The specific name is a noun in apposition shortened from the type locality.

Diagnosis.—Males of *E. guanato* sp. nov. ean be separated from all other related species by the distally hook-shaped dorsal braneh of the RTA (Fig. 136), and the lateral margin folded only towards the terminal end of the distal portion of the conductor (arrow in Fig. 136). Females are similar to speeimens of *E. gertschi, E. rothi, E. tlaxcala,* and *E. xilitla* sp. nov. but differ in having the posterior sclerite distinctly dumbbell-shaped (Figs. 130, 131; rather than posterior margin almost straight), and differ from *E. rothi* and *E. xilitla* sp. nov. in having the AME only as large as the PME, and differ from *E. gertschi* in having the distal segment of the PMS not twice as long as the basal segment (Fig. 129).

Description.—*Measurements:* Male (AB1127): CL 2.73, CW 2.2, STL 1.2, STW 1.22, OL 2.93, OW 1.83. Leg I (3.73, 0.92, 3.4, 3.7, 2.23), II (3.33, 0.87, 2.9, 3.47, 2.13), III (3.2, 0.8, 2.44, 3.37, 2.1), IV (3.7, 0.84, 3.56, 4.5, 2.4), Pedipalp (1.22, 0.42, 0.72, 1.1), bulbL 0.52. Female (holotype): CL 3.53, CW 2.6, STL 1.6, STW 1.58, OL 4.95, OW 3.75. Leg I (4.45, 1.35, 3.85, 3.9, 2.0), II (4.0, 1.2, 3.5, 3.73, 1.87), III (3.6, 1.15, 2.8, 3.6, 1.73), IV (4.7, 1.2, 4.05, 5.15, 2.0). Pedipalp (1.6, 0.733, 1.03, 1.47). EPL 0.33, EPW 0.58. Eyes: anterior eye row straight, posterior moderately procurved (Fig. 127). PME 0.19, PLE 0.21, AME 0.17, ALE 0.19. Eye distances: PME–PME 1 x PME, PME–AME 0.8–1 x PME, PME–PLE 1 x PME, PME–ALE 1.2 x PME, AME–AME 1 x AME, AME–ALE 0.5–0.75 x AME. CLY1 2–2.5 x AME, CLY2 1.2–1.5 x ALE. *Male pedipalp:* RTA with two branches, lateral branch simple, broadly lobe-like, dorsal branch distally hook-shaped (Fig. 136). Short dorsal spike on palpal tibia absent. Embolus length about 1.5 x CB, originating at 10 o'clock position, distal tip at 4 o'clock position. Conductor lamelliform, distal portion only moderately elongated, lateral margin only moderately folded towards terminal end (arrow in Fig. 136). Terminal end of conductor strongly elongated, pointed. Transversal ridge of conductor expressed as hyaline ridge, indistinct. Conductor membranously connected to tegulum. MA originating at 6–7 o'clock position, protruding, longer than wide, distal sclerite translucent, subtriangular, moderately pointed. MA membranously connected to tegulum. *Epigyne and vulva*: Epigynal hyaline area with anterior border m-shaped (Fig. 105). Posterior sclerite protruding anteroventrally as dumbbell-shaped moderately sclerotized bar (Figs. 130, 131), posterior membrane moderately protruding anteriad (Fig. 134). CO anterolateral to posterior selerite. Epigynal 'pseudo teeth' prominent (Fig. 131). Vulva consists of combined narrowly convoluted duct, CD less sclerotized, with distinet appendages, RC stronger selerotized (Figs. 133, 134). FD only represented by small, leaf-shaped appendages. *Other important characters*: Chelieeral promargin with 4, retromargin with7 small teeth, more proximally, the teeth become

smaller. Colulus developed as trapezoidal plate with distal margin moderately w-shaped. PMS bearing one conspicuously prominent spigot. PLS with distal segment longer than basal segment (4/3; Fig. 129). Tarsal trichobothria at leg I 8. Small teeth on paired claws of leg I 11. Leg spination: male pedipalp (3-0-0, 2-0-0, 1-1+1p-0), female pedipalp (2-0-0, 2-0-0, 2-1+1p-0), leg femora (1-3-1-0 or 1-1-3-0 in male, 1-2-1-0 or 1-2-3-0 in male, 1-1-1-0 or 1-4-3-0 in male, 1-0-1-0 or 1-2-1-0 in male), patellae (all 2-0-0), tibiae (0-0-0-0 or 1-0-0-0 or 0-0-0-1 in male, 1-0-0-0 or 1-1-0-1 in male, 2-1-0-1 or 2-2-2-3p in male, 2-1-0-1 or 2-2-2-1p+1+2p in male), metatarsi (0-0-0-4p+1 or 0-0-2-2p+2+1p+1 in male, 0-0-0-4p+1 or 0-2-0-4p+1 in male, 0-3-2-4p+1, 0-3-2-4p+1 or 0-4-4-1+4p+1 in male), tarsi (0, 0, 0-0-1-0 or 0-0-2-0 in male, 0-0-1-0 or 0-0-2-0 in male). *Coloration*: Carapace with two longitudinal symmetrieal dark bands, irregularly expressed, margins narrowly to broadly darkened (Fig. 126). Chelicerae frontally with indistinet dark patches (Fig. 127). Sternum darkened, with pale median band, midway interrupted, posteriorly with black patch (Fig. 128). Opisthosoma grayish with dark spots, without reddish pigments, indistinct grayish median band, anteriorly with dark fork-shaped patch, posteriorly with grayish chevrons (Fig. 126). Legs distinetly annulated. Colulus medially and laterally with dark patches. ALS basally darkened, basal segment of PLS darkened, distal segment of PLS only proximal half darkened.

Distribution.—Reported from four states of East-Central Mexico: Colima, Guanajuato, Jalisco and Michoacán.

Comments.—Male and female specimens were not collected together and show moderate morphological differences (e.g., size, leg spination) and are therefore only tentatively placed in the same species. The females collected close to Jacala and Coalcomán show some morphologieal differences to the type specimens. If these differences are part of intraspecific variation or a closely related taxa cannot satisfyingly be judged here. Therefore, the identification of these specimens remains uncertain.

Eratigena mexicana (Roth, 1968), comb. nov.
Figs. 138–149

Tegenaria flexuosa Roth, 1952: 285, figs. 1, 2 (in part, misidentified female).
Tegenaria mexicana Roth, 1968: 15, figs. 21–26.

Type material.—*Holotype male*. MEXICO: *Guerrero*: Taxco, in cave, 29 July 1956, V. Roth, W. Gertseh (AMNH).

Paratypes. 1 ♀ allotype, same data as holotype (AMNH); 2 ♂, same data (AMNH: AB1178); 5 ♂, same data except 28 July 1956 (AMNH: AB1154, AB1179); 1 ♂, Taxco, 1 October 1945, L. Isaacs (AMNH: AB1177).

Other material examined (of *E. mexicana* s. s.).—MEXICO: *Guerrero*: 1 ♂, Grutas de Acuitlapan, 10 mi. E of Taxco, 9 April 1968, W. Calvert (AMNH: AB1105); 1 ♂, 4 ♀, 1 juv., Taxco de Alarcón, E of Taxco, 1611 m, tropical forest, at stones and at rock faces near street (terrain break), 5 October 2014, A. Bolzern, E. González Santillán (NMB-ARAN-27511 to 27514: AB1217, AB1232, AB1247, AB1216: accession-nr. LN887153, LN887188); 4 ♀, 4 juv., same data except 1670 m, at rock faces near street (terrain break) (FC-UNAM: AB1206, AB1285); 1 ♀, same data except, W. of Colonia la Quebradora, 1825 m, secondary oak-pine forest, open, reddish stones, 1670 m (FC-UNAM: AB1273); 1 ♀, Taxco de Alarcón, Parque el Huixteco, 2434 m, pine-oak forest, 22 August 2013, H.E. León (NMB-ARAN-27515: AB1323). *México*: 1 ♀, Tenancingo, Tenancingo de Degollado, 2050 m, 7 October 1946, H. Wagner (AMNH: AB1129).

Other material examined (of *E.* cf. *mexicana*).—MEXICO: *Morelos*: 1 ♀, Cuernavaca, 1700 m, 1 September 1941, H. Wagner (AMNH: AB1128); 3 ♀, Cuernavaca, in shallow cave near town, 31 July 1956, W.J. Gertsch, V. Roth (AMNH: AB1123); 6 ♀, San Sebastian, north of Oaxtepec, 1874 m, old train tunnel, 16 December 2011, S. Huber (NMB-ARAN-27516: AB1091).

Diagnosis.—Male *E. mexicana* specimens differ from all Nearetic *Eratigena* speeies in having a distinctly elongated palpal femur and tibia (Fig. 140). Females are similar to *E. xilitla* sp. nov. and differ from other species in having the posterior membrane (internal posterior limitation of genital area) distinctly protruding anteriad (arrow in Fig. 148). They can be separated from *E. xilitla* sp. nov. specimens by having the posterior membrane without a notch and the AME diameter equal or smaller than that of the PME.

Description.—Essential information was provided by Roth (1968).

Distribution.—Reported from the states of Guerrero and México (Mexico). Reports from the state of Morelos are uncertain (see comment).

Comments.—The specimens from Morelos differ from typical *E. mexicana* specimens in having wider genital ducts (Fig. 149). Due to their affinity to *E. tlaxcala* (Figs. 203, 206) and the absence of males, the identification of these specimens remains uncertain.

Eratigena queretaro sp. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:853FD45F-18FF-4C3B-B2B4-F781C3CCC98F
Figs. 7, 150–161

Type material.—*Holotype female*. MEXICO: *Querétaro*: Pinal de Amoles, at road 120 between Jalpan de Serra and Pinal de Amoles, close to La Curva del Chuveje, 20 km W. of Jalpan, 1288 m, transitional forest, subtropical to mountainous, in tube with running water under road, 13 Oetober 2014, A. Bolzern, E. González Santillán (FC-UNAM: AB1302).

Paratypes. 1 ♂, 1 ♀, same data as holotype (FC-UNAM: AB1302).

Other material examined.—MEXICO: *Querétaro*: 3 ♀, Pinal de Amoles, at road 120 between Jalpan de Serra and Pinal de Amoles, close to Puerto del Perieo, 34 km W. of Jalpan, 2075 m, oak-pine forest, roadside cave, 14 October 2014, A. Bolzern, E. González Santillán (NMB-ARAN-27517: AB1258: aceession-nr. LN887154, LN887177); 1 ♀, Peñamiller, at path from Río Blanco to Cueva Puerto del Léon, 2014 m, oak forest, 14 October 2014, A. Bolzern, E. González Santillán (NMB-ARAN-27518: AB1218: accession-nr. LN887155, LN887178).

Etymology.—The specific name is a noun in apposition taken from the type locality.

Diagnosis.—Female specimens of *E. queretaro* sp. nov. differ from all others of the group in having an hourglass-shaped posterior sclerite (Figs. 154, 155), and an anteriorly distinctly arcuate genital duct with prominent appendages

(Figs. 157, 158). Males are similar to *E. decora, E. guanato* sp. nov., *E. rothi* and *E. selva* in having at least a moderately pointed distal sclerite of the MA (Figs. 159, 161). They differ from *E. guanato* sp. nov., *E. rothi* and *E. selva* in having a relatively slim, finger-shaped and simply pointed dorsal branch at the RTA (Fig. 161; rather than distally hook-shaped in *E. guanato* sp. nov., strong and basally bent in *E. rothi* and *E. selva*), and from *E. decora* in having a triangular and sharply pointed distal selerite of the MA (rather than subtriangular, moderately pointed).

Description.—*Measurements:* Male (paratype): CL 2.56, CW 1.9, STL 1.2, STW 1.18, OL 2.77, OW 1.44. Leg I (4.3, 1.0, 4.2, 4.65, 2.55), II (3.7, 0.93, 3.25, 3.85, 2.1), III (3.43, 0.84, 3.0, 4.03, 2.0), IV (4.35, 0.93, 4.03, 5.4, 2.6), Pedipalp (1.1, 0.46, 0.58, 0.86), bulbL 0.4. Female (holotype): CL 3.17, CW 2.33, STL 1.42, STW 1.3, OL 3.85, OW 2.5. Leg I (3.88, 1.2, 3.87, 3.83, 2.17), II (3.53, 1.17, 2.9, 3.3, 1.9), III (3.18, 1.0, 2.53, 3.2, 1.73), IV (4.0, 1.6, 3.75, 4.45, 2.23). Pedipalp (1.26, 0.5, 0.88, 1.3). EPL 0.38, EPW 0.58. Eyes: eye rows moderately procurved (Fig. 151). PME 0.15, PLE 0.17 , AME 0.14, ALE 0.2. Eye distances: PME–PME 0.8 x PME, PME–AME 0.8 x PME, PME–PLE 0.8–1 x PME, PME–ALE 1 x PME, AME–AME 0.6 x AME, AME–ALE 0.4 x AME. CLY1 1.75 x AME, CLY2 1 x ALE. *Male pedipalp:* RTA with two branches, lateral branch broadly lobe-like, indistinctly protruding, dorsal branch strongly sclerotized, narrowly finger shaped and sharply pointed (Figs. 160, 161). Short dorsal spike on palpal tibia absent. Embolus length about 1.5 x CB, originating at 9 o'clock position, distal tip at 3–4 o'clock position. Conductor lamelliform, distal portion only moderately elongated, lateral margin folded (Figs. 160, 161). Terminal end of conductor strongly elongated, pointed. Transversal ridge of conductor expressed as hyaline ridge. Conductor membranously connected to tegulum. MA originating at 6 o'clock position, protruding, longer than wide, distal sclerite translucent, black, long triangular, pointed (Figs. 159–161). MA membranously connected to tegulum. *Epigyne and vulva*: Epigyne medially with a pale, narrow oval hyaline area. Posterior sclerite protruding anteroventrally as moderately selerotized bar, hourglass-shaped, anterolaterally not rounded (Figs. 154, 155), posterior membrane protruding anteriad (Fig. 158). CO anterolateral to posterior sclerite. Epigynal 'pseudo teeth' minute (Figs. 154–156). Vulva consists of combined narrowly convoluted duet, anterior distinctly arcuate, with distinct appendages (Figs. 157, 158), RC stronger sclerotized. FD only represented by small, leaf-shaped appendages. *Other important characters*: Chelieeral promargin with 4, retromargin with 8 small teeth, more proximally, the teeth become smaller. Colulus developed as trapezoidal plate with distal margin moderately w-shaped. PMS bearing one conspicuously prominent spigot. PLS with distal segment longer than basal segment (3/2; Fig. 153). Trichobothria on cymbium and palpal tarsus absent. Tarsal trichobothria at leg I 7–8. Small teeth on paired claws of leg I 9–10. Leg spination: male pedipalp (2-0-0, 2-0-0, 1-1+1p-0), female pedipalp (2-0-0, 2-0-0, 2-1+1p-0), leg femora (0-2-0-0 or 2-2-0-0 or 2-3-2-0, 2-1-1-0 or 2-1-0-0 or 2-3-2-0, 2-1-1-0 or 2-2-2-0, 1-0-2-0 or 2-1-1-0), patellae (all 2-0-0), tibiae (0-0-0-1 or 1-0-0-2 or 1-1-0-3p, 2-1-0-0 or 2-1-0-3p+1, 2-1-1-1 or 2-2-2-2+1p, 2-1-1-2 or 2-2-2-3+1p), metatarsi (0-0-0-4p+1, 0-0-0-4p+1 or 0-1-0-4p+1, 0-2-2-4p+1 or 0-3-2-4p+1, 0-3-2-4p+1 or 0-3-3-1p+1+2p+1), tarsi (0, 0, 0, 0-0-1-0 or 0-1-3-0). *Coloration*: Carapace with two well expressed longitudinal symmetrical dark bands, margins narrowly to broadly darkened, head region dorsolaterally with two distinct, longitudinally curved dark bands (Fig. 150). Chelicerae frontally with indistinct dark patches (Fig. 151). Sternum darkened, with pale median band, midway interrupted (Fig. 152). Opisthosoma grayish with dark spots, median band with reddish pigments, anteriorly with dark patch, posteriorly with chevrons (Fig. 150). Legs annulated, ventrally more distinctly expressed as dorsally. Colulus moderately darkened. ALS basally and laterally darkened, basal segment of PLS distinctly darkened, distal segment of PLS only proximal half darkened.

Distribution.—Reported from only a narrow area in the state of Querétaro (Mexico).

Comments.—Specimens of *E. queretaro* sp. nov. were collected at three different sites in a relatively narrow area. The most natural habitat was at the path from Río Blanco to the Cueva Puerto del Léon in an old oak forest. There, in a shady valley, the spiders built their webs between rocks near a small creek. In contrast, representatives of this species were also collected in a tube with running water under a road (Fig. 6) within an area with subtropical to mountainous transitional forest.

Eratigena rothi (Gertsch, 1971), comb. nov.
Figs. 162–173

Tegenaria rothi Gertsch, 1971: 107, figs. 161–163.

Type material.—*Holotype male*. MEXICO: *Hidalgo*: Cueva de El Ocote, 1.5 km N. of Palomas, 20 July 1956, V. Roth, W.J. Gertsch (AMNH).

Other material.—MEXICO: *Hidalgo*: 1 ♂, 4 ♀, Jacala, collected in cave at night, 20 July 1956, V. Roth, W.J. Gertsch (AMNH: AB1173); 1 ♂, 5 ♀, same data except roadside cave, 1 mile N. of Palomas (AMNH: AB1157, AB1172); 3 ♀, 10–25 miles S. of Jacala, 1 July 1956, V. Roth, W. Gertsch (AMNH: AB1170); 1 ♂, 6 ♀, same data except 20 July 1956 (AMNH: AB1174, AB1175); 2 ♂, Tlanchinol, 43 km SW. of Huejutla, 1500 m, cloud forest, 14 August 1983, S. & J. Peck (AMNH: AB1138); 2 ♀, 2 km N. of del Pinalito, El Cardenal, 2301 m, coniferous forest, day catch, 16 October 2009, O. Francke, R. Paredes, J. Cruz, T. López, T. Palafox, C. Santibáñez, A. Valdez (CNAN: AB1201); 3 ♀, 7 juv., Zimapán, at street from Morelos (Trancas) to Puerto de Piedra, 2405 m, pine forest, at terrain break near street, at rocks, 10 October 2014, A. Bolzern, E. González Santillán (FC-UNAM: AB1211, AB1221, AB1270); 2 ♀, Nicolás Flores, elose to El Encinote, at street from Morelos (Trancas) to Puerto de Piedra, 2557 m, pine forest, at terrain break near street, at rocks, 10 October 2014, A. Bolzern, E. González Santillán (FC-UNAM: AB1257, AB1297); 1 ♀, same data except Puerto de Piedra, Santa Cueva, 2498 m, at rocks at cave entrance (NMB-ARAN-27519: AB1298: accession-nr. LN887156, LN887189); 3 ♂, 7 ♀, 4 juv., Chapulhuacán, between Puerto Chaballo and Chapuluacán, near road 85, 1087 m, in tube with running water under road, 10 October 2014, A. Bolzern, E. González Santillán (NMB-ARAN-27520 to 27524: AB1212, AB1228,

AB1241, AB1263, AB1210: accession-nr. LN887157, LN887190).

Diagnosis.—Male and female specimens of *E. rothi* are very similar to *E. xilitla* sp. nov. and differ from all other West Nearctic *Eratigena* species in having the AME larger than the PME. They differ from the sister species, *E. xilitla* sp. nov., in their larger size (CL longer than 4.5 mm; rather than smaller than 4 mm in *E. xilitla* sp. nov.). Males differ from *E. xilitla* sp. nov. by the shape of the distal sclerite of the MA (Fig. 167; long subtriangular and pointed, rather than spoon-shaped and rounded), and females differ by the shape of the posterior sclerite (Fig. 170).

Description.—Essential information was provided by Gertsch (1971).

Distribution.—Reported from several localities in the north-western part of the state of Hidalgo (Mexico).

Comments.—Morphologically, the two species *E. rothi* and *E. xilitla* sp. nov. are very closely related. This sister-species relationship is supported by the maximum likelihood tree based on mitochondrial sequences (Fig. 1). Specimens of *E. rothi* were collected during an excursion in 2014 from three different habitats: pine forest at terrain break, at a huge cave entrance between rocks and in a tube with running water under a road. *E. xilitla* sp. nov. seems to preferably inhabit oak-pine forests, but otherwise similar habitats.

Eratigena selva (Roth, 1968), comb. nov.
Figs. 174–194

Tegenaria mexicana selva Roth, 1968: 23, figs. 28, 29.
Tegenaria selva Roth: Gertsch, 1971: 105.

Type material.—*Holotype male.* MEXICO: *San Luis Potosí*: Cueva de la Selva, west of Xilitla on the Xilitla-Ahuacatlan road, north of Tamazunchale, 10 April 1966, T. Raines (AMNH).

Paratypes. 1 ♀ allotype, Sotano at Valle de los Fantasmas, 24 November 1966, J. Fish, J. Davis (AMNH: AB1131).

Other material examined.—MEXICO: *Hidalgo*: 1 ♂, Sótano de la Hoya de las Vigas, 1.5 km N Pinalito, 22 March 1981, J. Reddell, T. Archery (AMNH: AB1107); 1 ♀, La Sotono del Hondo de Pinalito, Hwy 85, 1 January 1976, C. Soileau, P. Strickkand (AMNH: AB1144). *San Luis Potosí*: 1 ♀, 5 km N Valle de Los Fantasmas, 40 km E Zaragoza, Cueva de la Laguna, 3000 m, 20 May 1972, Wm. Elliott, P. Lynn, R.M. McEachern (AMNH: AB1101); 1 ♂, Cueva Pueblo Ilamado, 58 km Mpio Villa de Zaragoza, 2298 m, pine-oak forest, date unknown, A. Valdez, O. Francke, J. Cruz, C. Santibáñez (CNAN: AB1197); 1 ♀, Gruta de los Muertes, Xilitla Plateau, 28 March 1900, D. Pate (AMNH: AB1158); 3 ♀, Sotana de La Silleta, 30 March 1980, D. Honea (AMNH, AB1109); 2 ♀, Municipio de Zaragoza, Cueva de los Caballos, 30 km E San Luis Potosí, 3000 m, 18 May 1972, Wm. Elliott (AMNH: AB1146); 4 ♀, Sierra del Pina, Microwave Tower Road, La Cueva de los Murcielagos, 29 December 1975, C. Soileau, P. Strickkand (AMNH: AB1147); 1 ♀, Sotano de Abernathy, W. of Valle de los Fantasmos, 30 January 1969, Wm. Elliott, D. Honea, M. Abernathy (AMNH: AB1160); 1 ♀, Sotano de Arañas, W of Valle de los Fantasmas, 29 January 1969, R. Harmon, J. Cepeda (AMNH: AB1132); 1 ♂, 3 ♀, Sotano de Golondrina, Puerto Altamira, 40 km E San Luis Potosi, 3000 m, 17 March 1972, Wm. Elliott, R. Mitchell, J.A.L. Cooke, G. Campell, G. Graves, M. Brownfield (AMNH: AB1151); 2 ♂, 1 ♀, Sotano de Las Golondrinas, Valle de los Fantasmos, 29 November 1968, Wm. Elliott, J. Jarl, S. Cathey, M. Burk (AMNH: AB1159); 2 ♀, Sotano de Ojo de Agua, 4 km S San Francisco, 30 November 1968, Wm. Elliott, J. Jarl (AMNH: AB1133); 2 ♀, Sotano Puerto de los Lobos, San Francisco, 14 September 1968, B. Elliott (AMNH: AB1134).

Diagnosis.—Male and female specimens of *E. selva* differ from all West Nearctic *Eratigena* species by their large size in combination with very long legs (CL > 5 mm, patella-tibia length leg I > 9 mm) and an indistinctly patterned abdomen (grayish). In addition, females differ by having a very strongly sclerotized epigynal posterior sclerite (Figs. 182, 191).

Description.—Essential information was provided by Roth (1968).

Distribution.—Reported from the north-western part of the state of Hidalgo and the state of San Luis Potosí (Mexico).

Comments.—Even though the type locality of *E. selva* (Cueva de la Selva, now called Cueva del Salitre) was revisited during an excursion in October 2014, no specimens or webs could be detected there.

The specimens from Sotano de Golondrina (AB1151) are morphologically very similar to typical *E. selva* specimens, but differ to some extent in epigynal and genital morphology (Figs. 186–188, 191–194). However, due to the lack of more information (more specimens with these features, DNA data) these specimens are here treated as a variation of *E. selva*.

Eratigena tlaxcala (Roth, 1968), comb. nov.
Figs. 195–206

Tegenaria mexicana tlaxcala Roth, 1968: 24.
Tegenaria tlaxcala Roth: Brignoli, 1974: 230.

Type material.—*Holotype male.* MEXICO: *Tlaxcala*: Tlaxcala, in underground water conduit, 26 July 1956, V. Roth, W. Gertsch (AMNH).

Paratypes. 1 ♀ allotype, same data as holotype (AMNH); 3 ♂, 1 ♀, same data (AMNH: AB1171).

Other material examined (of *E. tlaxcala* s. s.).—MEXICO: *Distrito Federal*: 1 ♀, Gustavo A. Madero, cañada hacia la cueva del Fraile, 2614 m, 25 June 2009, H. Montaño, A. Valdez, R. Paredes, T. Garrido (CNAN: CNAN-Ar009746). *México*: 1 ♂, 58 km E. of Mexico City, pine forest, under log, 24 July 1956, V. Roth, W. Gertsch (AMNH: AB1162). *Puebla*: 1 ♂, Huachinango, 4.4 miles SW. of Huachinango, 1700 m, moist ravine oak forest, malt traps, 25 July 1969, collector unknown (AMNH: AB1110); 1 ♂, pyramid at Cholula, 20 August 1965, J. Reddell, J. Fish, W. Bell (AMNH: AB1113); 1 ♀, San Pedro Cholula, at northern slope of hill NE. of Cholula, 2221 m, pine forest, small cave, 8 October 2014, A. Bolzern, E. González Santillán (NMB-ARAN-27525: AB1271: accession-nr. LN887159, LN887191); 1 ♀, W. of Rio Frio, 2956 m, 22 August 1964, W. & J. Ivie (AMNH: AB1111). *Tlaxcala*: 5 ♀, 2 juv., Tlaxcala, Ocatlán, Barage Chapitel, within city in an open space between houses, 2222 m, old manmade tunnel/cave under the city, at the entrance (which was a dump), 9 October 2014, A. Bolzern, E. González Santillán (FC-UNAM: AB1229, AB1242, AB1296; NMB-ARAN-27526: AB1233: accession-nr. LN887158, LN887192).

Other material examined (of *E.* cf. *tlaxcala*).—MEXICO: *Guerrero*: 1 ♂, Tetipan, 5 km E de Casahuates, 2275 m, pine-oak forest, 4 June 2010, O. Francke, D. Barrales, J. Cruz, A. Valdez (CNAN: AB1196).

Diagnosis.—Males of *E. tlaxcala* are similar to specimens of *E. caverna*, *E. fernandoi* sp. nov., *E. gertschi*, *E. selva* (variation) and *E. xilitla* sp. nov. and differ from other species in having a spoon-shaped, distally rounded distal sclerite (Fig. 195,197; rather than subtriangular or triangular and distally pointed). Males and females differ from *E. caverna* and *E. xilitla* sp. nov. in having normally developed eyes with the AME as large as the PME (Fig. 199; rather than all reduced in *E. caverna*, PME larger than AME in *E. xilitla* sp. nov.). Males differ from *E. fernandoi* sp. nov. and *E. gertschi* in having a narrow, distinctly longer then wide distal sclerite of the MA (Fig. 197; rather than broad), and from *E. selva* (variation) in having a conductor with an unevenly curved distal margin (arrow in Fig. 196; rather than evenly curved). Females are very similar to *E. gertschi, E. guanato* sp. nov., *E. rothi* and *E. xilitla* sp. nov., but differ from *E. rothi* and *E. xilitla* sp. nov. in having the AME as large as the PME, differ from *E. guanato* sp. nov. in having the posterior sclerite not distinctly dumbbell-shaped, and differ from *E. gertschi* in having the distal segment of the PLS not twice as long as the basal segment.

Description.—Essential information was provided by Roth (1968).

Distribution.—Reported from three states: Distrito Federal, Puebla and Tlaxcala (Mexico).

Comments.—The specimen from Guerrero differs to some extent morphologically from typical *E. tlaxcala* specimens (e.g. shape of RTA and median apophysis) and its identification remains doubtful.

Eratigena xilitla sp. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:A9F274FD-3456-4D7F-8A21-B9DF2B9B5DB8
Figs. 207–218

Type material.—*Holotype male*. MEXICO: *San Luis Potosí*: Xilitla, at road from Xilitla to Las Adjuntas, Cueva del Aqua, 451 m, near cave entranee at rock surfaces, 12 October 2014, A. Bolzern, E. González Santillán (FC-UNAM: AB1219: accession-nr. LN887161, LN887180)..

Paratypes. 1 ♀, 2 juv., same data as holotype (FC-UNAM: AB1219: accession-nr. LN887161, LN887180); 1 ♀, 4 juv., same data (NMB-ARAN-27527: AB1301).

Other material.—MEXICO: *Hidalgo*: 2 ♀, 2.5 km N. of junction Zacualtipan-Santiago Tianguistengo, 2101 m, pine forest, 6 November 2010, A. Valdez, O. Francke, J. Cruz, C. Santibáñez, E. Miranda (CNAN: AB1195). *Puebla*: 2 ♀, 1 juv., Ajalpan, street between Pala and Nicolás Bravo, 2653 m, oak-pine forest, at terrain break at steep slope near path in the forest, 7 October 2014, A. Bolzern, E. González Santillán (NMB-ARAN-27528 to 27529: AB1278, AB1287: accession-nr. LN887160, LN887179); 1 ♀, same data except 2619 m (FC-UNAM: AB1246). *San Luis Potosí*: 1 ♀, Tamazunchale, 6 July 1941, L.I. Davis (AMNH: AB1125). *Querétaro*: 1 ♀, 2 juv., Pinal de Amoles, at road 120 between Jalpan de Serra and Pinal de Amoles, close to La Curva del Chuveje, 20 km W. of Jalpan, 1288 m, transitional forest, subtropical to mountainous, in tube with running water under road, 13 October 2014, A. Bolzern, E. González Santillán (FC-UNAM: AB1235). *Veracruz*: 1 ♂, 4 ♀, 10 miles W. of Jalapa, volcanic cave in pine forest, 26 July 1956, V. Roth, W. Gertseh (AMNH: AB1164); 1 ♀, Acejete, 1 km NE. of La Joya, 2204 m, 30 October 2006, O. Francke, A. Valdez, C. Santibáñez (CNAN: AB1194); 1 ♂, 1 ♀, 2 juv., Coatepec, village park, 1265 m, 10 December 2010, S. Huber (NMB-ARAN-27530: AB1090); 1 ♂, Huatusco, 7 km E. of Huatusco, cloud forest, 22 June 1983, S. & J. Peck (AMNH: AB1136).

Etymology.—The specific name is a noun in apposition taken from the type locality.

Diagnosis.—Male and female specimens of *E. xilitla* sp. nov. are very similar to *E. rothi* and differ from all other West Nearctic *Eratigena* species in having the AME larger than the PME. They differ from the sister species, *E. rothi*, in their smaller size (CL shorter 4 mm; rather than longer than 4.5 mm in *E. rothi*). Males differ from *E. rothi* sp. nov. by the shape of the distal sclerite of the MA (Figs. 207, 209; spoon-shaped and rounded, rather than long subtriangular and pointed), and females differ by the shape of the posterior sclerite (Fig. 215).

Description.—*Measurements:* Male (holotype): CL 3.13, CW 2.47, STL 1.44, STW 1.36, OL 3.37, OW 1.7. Leg I (5.48, 1.33, 5.35, 6.15, 3.15), II (4.4, 1.1, 4.15, 4.95, 2.65), III (4.0, 1.0, 3.33, 4.5, 2.25), IV (5.35, 1.16, 4.75, 6.5, 3.33), Pedipalp (1.6, 0.54, 0.82, 1.36), bulbL 0.5. Female (paratype): CL 3.17, CW 2.4, STL 1.4, STW 1.36, OL 4.0, OW 2.9. Leg I (4.2, 1.1, 3.85, 4.0, 2.15), II (3.45, 1.08, 2.83, 3.1, 1.75), III (3.3, 1.0, 2.47, 3.1, 1.63), IV (4.22, 1.03, 3.5, 4.2, 2.03). Pedipalp (1.4, 0.54, 0.88, 1.32). EPL 0.31, EPW 0.67. Eyes: eye rows moderately procurved (Fig. 212). PME 0.16, PLE 0.18, AME 0.18, ALE 0.18. Eye distances: PME–PME 0.8 x PME, PME–AME 1 x PME, PME–PLE 1 x PME, PME–ALE 1.3 x PME, AME–AME 0.3 x AME, AME–ALE 0.3 x AME. CLY1 1.2 x AME, CLY2 1 x ALE. *Male pedipalp:* RTA with two branches, lateral branch subtriangular lobe-like, moderately ventrad protruding, dorsal branch strongly finger shaped, distally truncated (Fig. 209). Short dorsal spike on palpal tibia absent. Embolus length about 1.4 x CB, originating at 9–10 o'clock position, distal tip at 3 o'clock position. Conductor lamelliform, distal portion only moderately elongated, lateral margin folded. Terminal end of conductor strongly elongated, pointed. Transversal ridge of conductor expressed as indistinct hyaline ridge. Conductor membranously connected to tegulum. MA originating at 6 o'clock position, protruding, longer than wide, distal sclerite spoon-shaped, rounded (Figs. 207–209). MA membranously connected to tegulum. *Epigyne and vulva*: Epigyne medially with a pale, hyaline area, long oval. Posterior sclerite protruding anteroventral as moderately sclerotized bar, dumbbell-shaped (Fig. 215), posterior membrane strongly protruding anteriad and notched (Fig. 218). CO anterolateral to posterior sclerite. Epigynal 'pseudo teeth' prominent (Fig. 215). Vulva consists of combined narrowly convoluted duct, CO less sclerotized with distinct appendages (Figs. 217, 218). FD only represented by small, leaf-shaped appendages. *Other important characters*: Cheliceral promargin with 4–5, retromargin with 7–11 small teeth, more proximally, the teeth become smaller. Colulus developed as trapezoidal plate with distal margin moderately w-shaped. PMS bearing one conspicuously prominent spigot. PLS with distal segment

longer than basal segment (3/2; Fig. 214). Trichobothria on cymbium and palpal tarsus absent. Tarsal trichobothria at leg I 8. Small teeth on paired claws of leg I 8–9. Leg spination: male pedipalp (3-0-0 or 3-1-0, 2-0-0, 1-1+1p-0), female pedipalp (2-0-0, 2-0-0, 2-1+1p-0), leg femora (1-3-2-0 or 2-3-2-0, 1-3-2-0 or 2-3-2-0, 1-2-2-0, 1-2-2-0 or 2-1-1-0), patellae (all 2-0-0), tibiae (1-1-0-0, or 1-1-0-1, 1-1-0-0, 2-1-1-1, 2-2-1-1 or 2-2-2-1 or 2-2-2-2), metatarsi (0-0-0-1p+1+2p+1, 0-1-0-1p+1+2p+1, 0-2-2-1p+1+2p+1 or 0-3-2-4p+1, 0-3-2-4p+1 or 0-3-3-4p+1), tarsi (0, 0, 0-0-1-0 or 0-0-2-0, 0-1-2-0 or 0-1-3-0). *Coloration*: Carapace with two broad longitudinal symmetrical dark bands, margins narrowly to broadly darkened (Fig. 211). Sternum darkened, with pale median band with moderately serrated lateral margins (sometimes indistinct), posteriorly with indistinct black patch (Fig. 213). Opisthosoma dark brown, with distinct reddish median band with black spots, bordered by yellowish bands, posteriorly with yellowish chevrons (Fig. 211). Legs distinctly annulated, dorsally sometimes indistinct. Colulus laterally with dark patches. ALS basally and distally darkened, basal segment of PLS darkened, distal segment of PLS only proximal third darkened (Fig. 214).

Distribution.—Reported from five states along the eastern mountain range (Sierra Madre Oriental): Hidalgo, Puebla, Querétaro, San Luis Potosí, and Veracruz (Mexico).

Comments.—The specimens collected by Roth and Gertseh in 1956 were misidentified as *T. tlaxcala* (Roth, 1968: 25). See also comment for *E. rothi*.

DISCUSSION

The result that the endemic species in the Nearctic region belong to both genera, *Tegenaria* and *Eratigena*, is surprising. This hypothesis would imply that either both genera originated earlier than the split of Laurasia (approximately 55 million years ago; Ellis & Stoker 2014), or that one or both of them invaded the continent later.

The mexican *Eratigena* species can be split into the two described, well diagnosable groups: the *flexuosa*-group and the *mexicana*-group. Based on morphological data and mtDNA sequences, the *mexicana*-group is more complex with some very elosely related species. Differences in male and female genitalia between species are sometimes hard to detect. That is why we even propose to use size as a character to separate species (Bolzern et al. 2013a). However, most species are well diagnosed by morphological and molecular data, although some specimen identifications remain uncertain due to the low number of available individuals (unknown magnitude of intraspecific variation) and the absence of molecular data for certain species.

In addition to the species groups in foeus, it is mentionable that (based on mtDNA sequences; Fig. 1) *Textrix denticulata* (Olivier, 1789) is placed outside *Textrix* Sundevall, 1833, that *Agelena canariensis* Lucas, 1838 is closely related to *Agelescape gideoni* Levy, 1996 (and only distantly related to other *Agelena* Walckenaer, 1805 species), and that the *Novalena* Chamberlin & Ivie, 1942 specimens from Mexico represent a monophyletic clade apart from *Novalena intermedia* (Chamberlin & Gertsch, 1930).

ACKNOWLEDGMENTS

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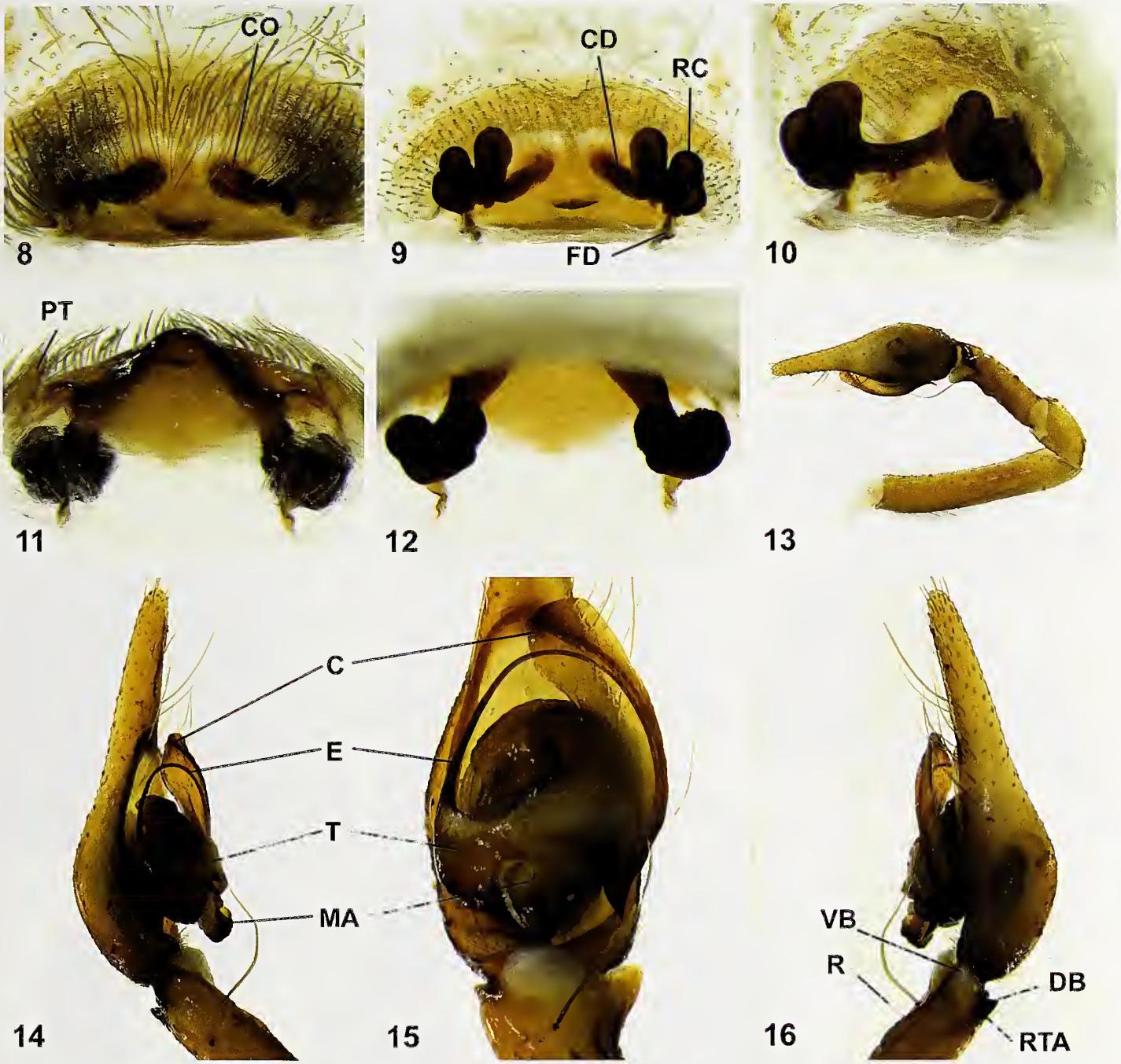


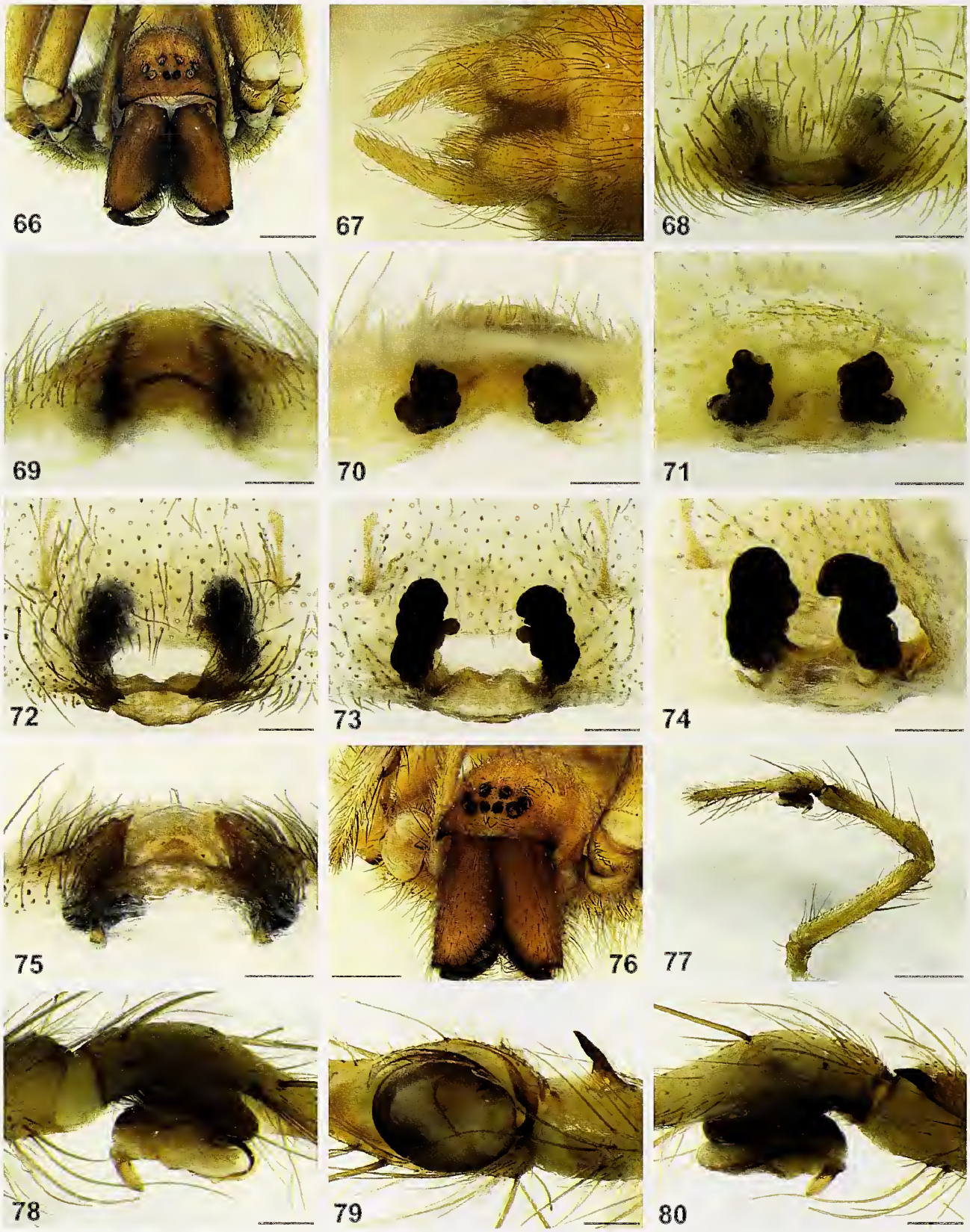
Figures 8–16.—*Tegenaria chiricahuae* Roth, 1968, female (8–12) and male (13–16). 8, epigynal area, ventral view; 9, vulva, dorsal view; 10, same, dorsolateral view; 11, same, posterior view; 12, same, anterior view; 13, left male pedipalp, dorsoretrolateral view; 14, cymbium and bulb, prolateral view; 15, same, ventral view; 16, same, retrolateral view. C = conductor; CD = copulatory duct; CO = copulatory opening; DB = dorsal branch of RTA; E = embolus; FD = fertilization duct; PT = epigynal 'pseudo teeth'; MA = median apophysis; R = lateroventral ridge of RTA; RC = receptaculum; RTA = retrolateral tibial apophysis; T = tegulum; VB = ventral branch of RTA.

Figures 17–29.—*Eratigena edmundoi* sp. nov., female (17–22) and male (23–29). 17, habitus, dorsal view; 18, epigynal area, ventral view; 19, vulva, dorsal view; 20, same, posterior view; 21, same, anterior view; 22, same, dorsolateral view; 23, carapace and chelicerae, anterior view; 24, sternum ventral view; 25, spinnerets, ventral view; 26, bulb, prolateral view; 27, same, ventral view; 28, same, retrolateral view; 29, left male pedipalp, retrolateral view. Scale of 17 = 2 mm; scale of 24 = 1 mm; scale of 23 = 0.5 mm; scale of other images = 0.2 mm. CD = copulatory duct; DP = distal portion of conductor; DS = distal sclerite at MA; FD = fertilization duct; PM = Posterior membrane; PS = posterior sclerite; PT = epigynal 'pseudo teeth'; RC = receptaculum; TR = transversal ridge at conductor.

Figures 30–41.—*Eratigena flexuosa* (F.O. Pickard-Cambridge, 1902), male holotype (30–36) and female (37–41). 30, habitus, dorsal view; 31, sternum, ventral view; 32, spinnerets, ventral view; 33, cymbium and bulb, prolateral view; 34, same, ventral view; 35, same, retrolateral view; 36, chelicerae, posterior view; 37, carapace and chelicerae, anterior view; 38, epigynal area, ventral view; 39, vulva, posterior view; 40, same, anterior view; 41, same, dorsal view. Scale of 30 = 2 mm; scale of 31, 37 = 1 mm; scale of 32, 36 = 0.5 mm; scale of other images = 0.2 mm.

Figures 42–53.—*Eratigena florea* (Brignoli, 1974), female (42–46) and male (47–53). 42, epigynal area, ventral view; 43, vulva, anterior view; 44, same, dorsolateral view; 45, same, dorsal view; 46, same, variation (specimen labeled "prope" *florea* by Brignoli); 47, habitus, dorsal view; 48, carapace and chelicerae, anterior view; 49, sternum, ventral view; 50, spinnerets, ventral view; 51, cymbium and bulb, prolateral view; 52, same, ventral view; 53, same, retrolateral view. Scale of 47 = 2 mm; scale of 48–49 = 1 mm; scale of 50 = 0.5 mm; scale of other images = 0.2 mm.

Figures 54–65.—*Eratigena yarini* sp. nov., female holotype (54–62) and male paratype (63–65). 54, habitus, dorsal view; 55, carapace and chelicerae, anterior view; 56, sternum, ventral view; 57, spinnerets, ventral view; 58, epigynal area, ventral view; 59, vulva, anterior view; 60, same, dorsolateral view; 61, same, dorsal view; 62, same, posterior view; 63, cymbium and bulb, prolateral view; 64, same, ventral view; 65, same, retrolateral view. Scale of 54 = 2 mm; scale of 55–56 = 1 mm; scale of 57 = 0.5 mm; scale of other images = 0.2 mm.

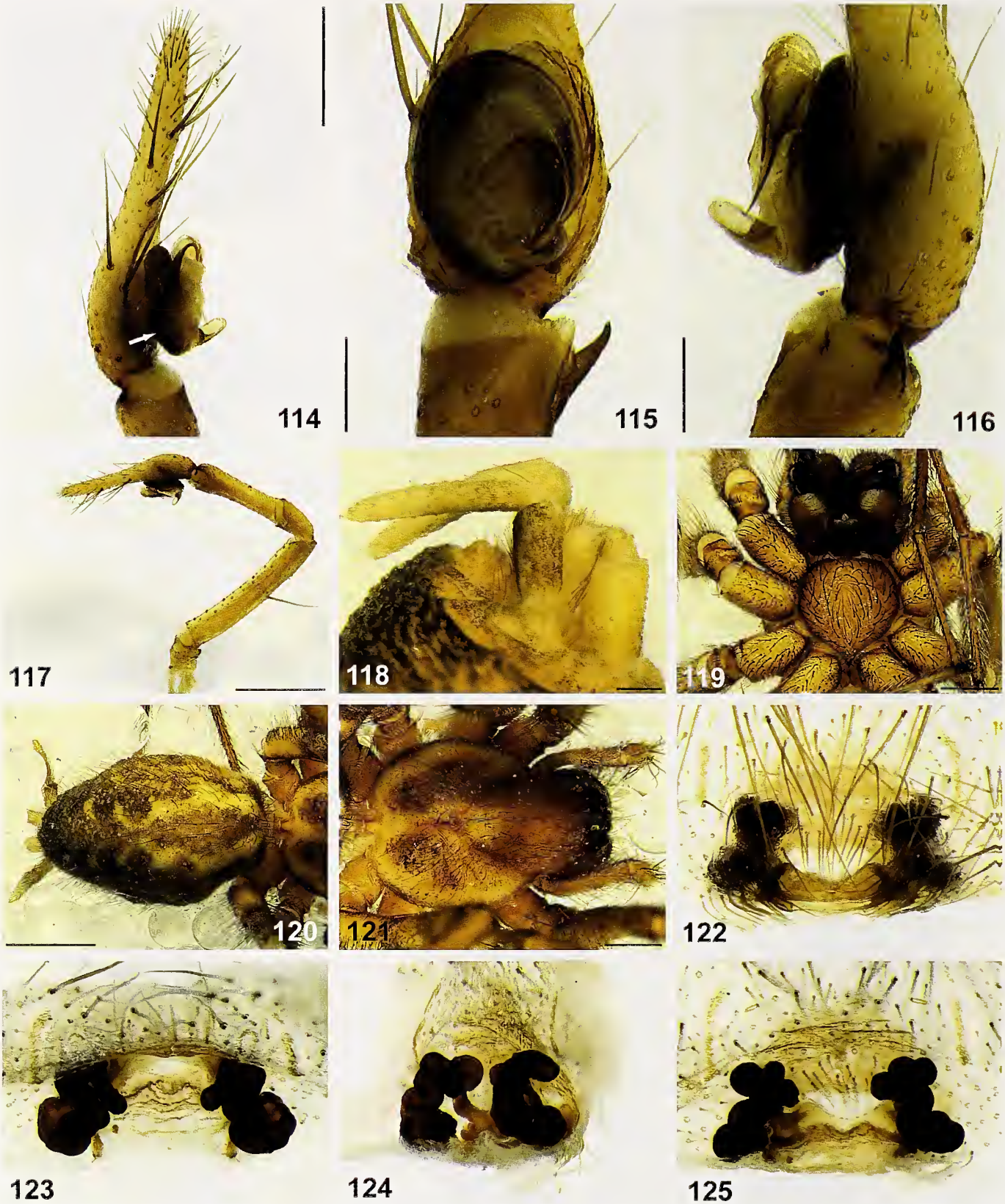


Figures 66–80.—*Eratigena blanda* (Gertsch, 1971), female holotype (66–71), and *Eratigena* cf. *gertschi*, female (72–75), and male (76–80). 66, carapace and chelicerae, anterior view; 67, spinnerets, ventral view; 68, epigynal area, ventral view; 69, vulva, posterior view; 70, same, anterior view; 71, same, dorsal view; 72, epigynal area, ventral view; 73, vulva, dorsal view; 74, same, dorsolateral view; 75, same, posterior view; 76, carapace and chelicerae, anterior view; 77, pedipalp, retrolateral view; 78, bulb, prolateral view; 79, same, ventral view; 80, same, retrolateral view. Scale of 66, 76–77 = 1 mm; scale of 67 = 0.5 mm; scale of other images = 0.2 mm.

Figures 81–89.—*Eratigena caverna* (Gertsch, 1971), male holotype (81–84) and female allotype (85–89). 81, bulb, prolateral view; 82, same, ventral view; 83, same, retrolateral view; 84, carapace and chelicerae, anterior view; 85, epigynal area, ventral view; 86, vulva, posterior view; 87, same, dorsolateral view; 88, same, dorsal view; 89, same, anterior view. Scale of 84 = 1 mm; scale of other images = 0.2 mm.

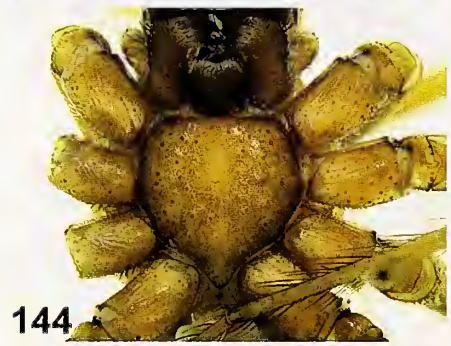
Figures 90–101.—*Eratigena decora* (Gertsch, 1971), male holotype (90–96) and female paratype (97–101). 90, habitus, dorsal view; 91, carapace and chelicerae, anterior view; 92, spinnerets, ventral view; 93, bulb, prolateral view; 94, same, ventral view; 95, same, retrolateral view; 96, pedipalp, retrolateral view; 97, epigynal area, ventral view; 98, vulva, posterior view; 99, same, anterior view; 100, same, dorsal view; 101, same, dorsolateral view. Scale of 90 = 2 mm; scale of 91, 96 = 1 mm; scale of other images = 0.2 mm.

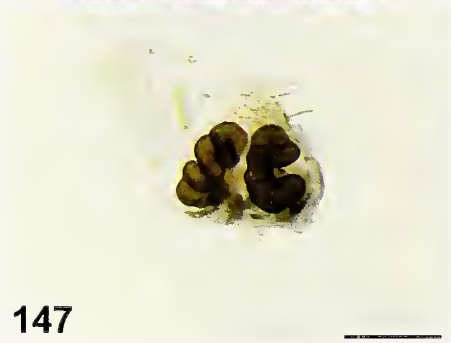
Figures 102–113.—*Eratigena fernandoi* sp. nov., female paratype (102–108) and male holotype (109–113). 102, carapace and chelicerae, anterior view; 103, sternum, ventral view; 104, spinnerets, ventral view; 105, epigynal area, ventral view; 106, vulva, dorsal view; 107, same, anterior view; 108, same, posterior view; 109, habitus, dorsal view; 110, pedipalp, retrolateral view; 111, bulb, prolateral view; 112, same, ventral view; 113, same, retrolateral view. Scale of 109 = 2 mm; scale of 102–103 = 1 mm; scale of 104, 110 = 0.5 mm; scale of other images = 0.2 mm.



Figures 114–125.—*Eratigena gertschi* (Roth, 1968), male holotype (114–118) and female (119–125). 114, cymbium and bulb, prolateral view; 115, bulb, ventral view; 116, same, retrolateral view; 117, pedipalp, same; 118, spinnerets, lateral view; 119, sternum, ventral view; 120, abdomen, dorsal view; 121, carapace, same; 122, epigynal area, ventral view; 123, vulva, anterior view; 124, same, dorsolateral view; 125, same, dorsal view. Scale of 120 = 2 mm; scale of 117, 119 = 1 mm; scale of 114 = 0.5 mm; scale of other images = 0.2 mm.

Figures 126–137.—*Eratigena guanato* sp. nov., female paratype (126–134), and male (135–137). 126, habitus, dorsal view; 127, carapace and chelicerae, anterior view; 128, sternum, ventral view; 129, spinnerets, ventral view; 130, epigynal area, ventral view; 131, same; 132, vulva, anterior view; 133, same, dorsolateral view; 134, same, dorsal view; 135, bulb, prolateral view; 136, same, ventral view; 137, same, retrolateral view. Scale of 126 = 2 mm; scale of 127–128 = 1 mm; scale of other images = 0.2 mm.



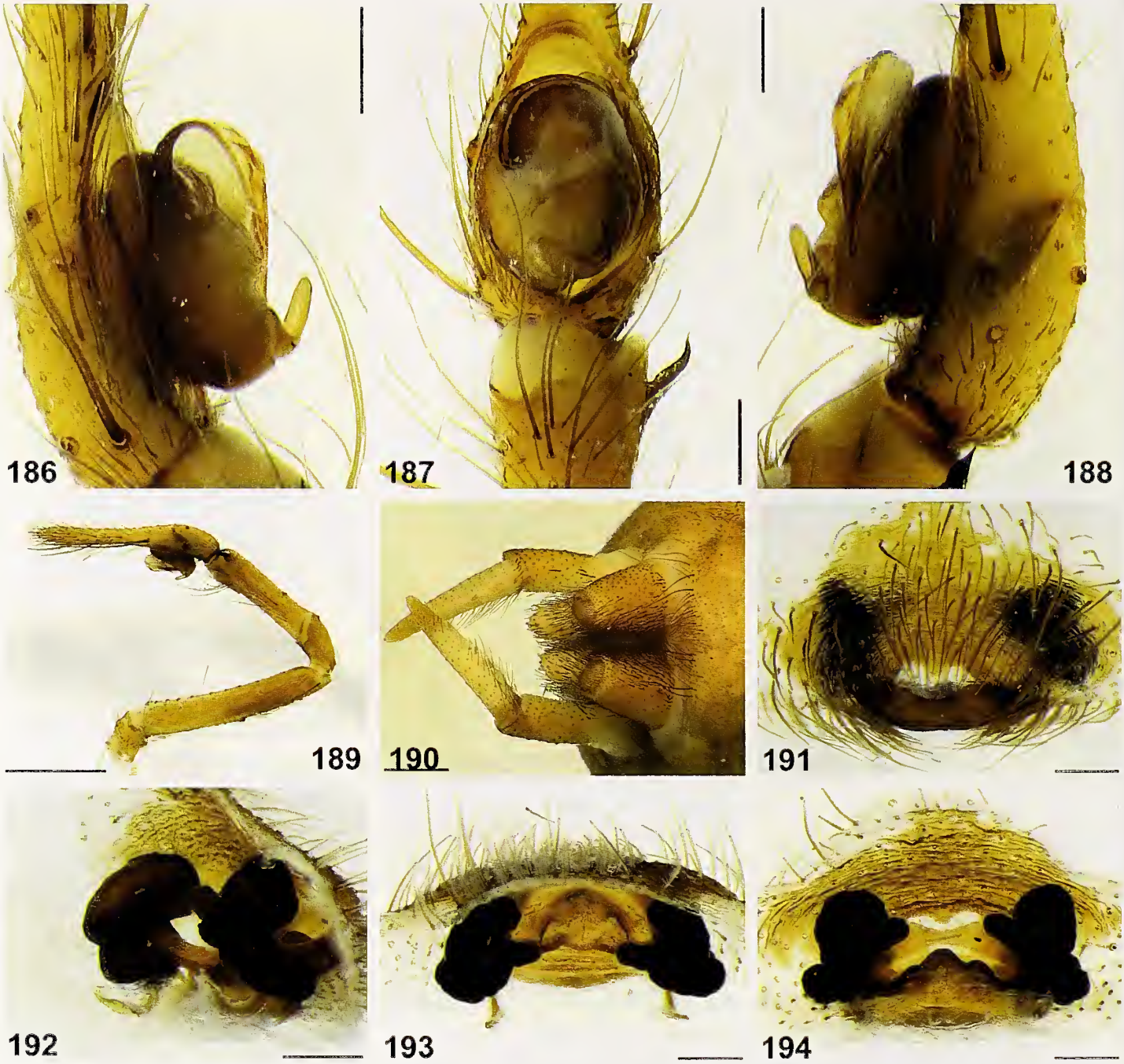


Figures 138–149.—*Eratigena mexicana* (Roth, 1968), male holotype (138–143) and female (144–148). *Eratigena* cf. *mexicana*, female (149) . 138, carapace and chelicerae, anterior view; 139, abdomen, dorsal view; 140, pedipalp, retrolateral view; 141, bulb, prolateral view; 142, same, ventral view; 143, same, retrolateral view; 144, sternum, ventral view; 145, epigynal area, ventral view; 146, vulva, anterior view; 147, same, dorsolateral view; 148, same, dorsal view; 149, same. Scale of 139–140 = 2 mm; scale of 138, 144 = 1 mm; scale of other images = 0.2 mm.

Figures 150–161.—*Eratigena queretaro* sp. nov., female holotype (150–153, 155–158), female paratype (154), and male paratype (159–161). 150, habitus, dorsal view; 151, carapace and chelicerae, anterior view; 152, sternum, ventral view; 153, spinnerets, ventral view; 154–155, epigynal area, ventral view; 156, vulva, posterior view; 157, same, anterior view; 158, same, dorsal view; 159, bulb, prolateral view; 160, same, ventral view; 161, same, retrolateral view. Scale of 150 = 2mm; scale of 151–152 = 1 mm; scale of 153 = 0.5 mm; scale of other images = 0.2 mm.

Figures 162–173.—*Eratigena rothi* (Gertsch, 1971), male holotype (162–169), and female (170–173). 162, abdomen, dorsal view; 163, carapace, same; 164, carapace and chelicerae, anterior view; 165, cymbium and bulb, prolateral view; 166, bulb, ventral view; 167, same, retrolateral view; 168, sternum, ventral view; 169, spinnerets, ventral view; 170, epigynal area, same; 171, vulva, anterior view; 172, same, posterior view; 173, same, dorsal view. Scale of 162–164, 168 = 1 mm; scale of 165, 169 = 0.5 mm; scale of other images = 0.2 mm.

Figures 174–185.—*Eratigena selva* (Roth, 1968), male holotype (174–178), female (AB1158, 179–185). 174, bulb, prolateral view; 175, same, ventral view; 176, same, retrolateral view; 177, carapace and chelicerae, anterior view; 178, pedipalp, retrolateral view; 179, sternum, ventral view; 180, abdomen, dorsal view; 181, spinnerets, ventral view; 182, epigynal area, ventral view; 183, vulva, posterior view; 184, same, anterior view; 185, same, dorsal view. Scale of 177–178, 180 = 2 mm; scale of 179 = 1 mm; scale of 181 = 0.5 mm; scale of other images = 0.2 mm.



Figures 186–194.—Variation of *Eratigena selva* (Roth, 1968), male (186–189), and female (190–194) from Sotano de Golondrina (AB1151). 186, bulb, prolateral view; 187, same, ventral view; 188, same, retrolateral view; 189, pedipalp, same; 190, spinnerets, ventral view; 191, epigynal area, ventral view; 192, same, dorsolateral view; 193, same, anterior view; 194, same, dorsal view. Scale of 189 = 1 mm; scale of 190 = 0.5 mm; scale of other images = 0.2 mm.

Figures 195–206.—*Eratigena tlaxcala* (Roth, 1968), male holotype (195–197), and female (198–207). 195, bulb, prolateral view; 196, same, ventral view; 197, same, retrolateral view; 198, abdomen, dorsal view; 199, carapace and chelicerae, anterior view; 200, sternum, ventral view; 201, spinnerets, ventral view; 202, epigynal area, ventral view; 203, vulva, dorsal view; 204, same, posterior view; 205, same, anterior view; 206, same, variation, dorsal view. Scale of 198, 200 = 2 mm; scale of 199 = 1 mm; scale of 201 = 0.5 mm; scale of other images = 0.2 mm.

Figures 207–218.—*Eratigena xilitla* sp. nov., male holotype (207–210), and female paratype (211–218). 207, bulb, prolateral view; 208, same, ventral view; 209, same, retrolateral view; 210, pedipalp, same; 211, habitus, dorsal view; 212, carapace and chelicerae, anterior view; 213, sternum, ventral view; 214, spinnerets, same; 215, epigynal area, same; 216, vulva, posterior view; 217, same, anterior view; 218, same, dorsal view. Scale of 211 = 2 mm; scale of 212–213 = 1 mm; scale of 210, 214 = 0.5 mm; scale of other images = 0.2 mm.

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The Mediterranean recluse spider *Loxosceles rufescens* (Dufour, 1820) (Araneae: Sicariidae) established in a natural cave in Thailand

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Abstract. *Loxosceles rufescens* (Dufour, 1820), the Mediterranean recluse spider, is a cosmopolitan species with toxic venom which can occasionally cause dermatological injuries in humans. Here, we report the finding of *L. rufescens* through intensive survey and exploration of six natural limestone caves in the western region of Thailand. These data provide the first direct evidence of *L. rufescens* living in large numbers in a natural habitat outside of their native Mediterranean range. Although the currently known distribution of *L. rufescens* in Thailand is quite narrow (the spiders were only found in one of the six caves explored), data on their biology and local habitat preferences are provided to better understand the colonization requirements of this species in the target area.

Keywords: Cave, distribution, toxic, violin spider

The genus *Loxosceles* Heineken & Lowe, 1832, the recluse or violin spiders, is one of two genera in the family Sicariidae (Araneae), comprising 107 species distributed around the world (Platnick 2015). They are notorious for their bites, which occasionally produce a suite of symptoms known as 'loxoscelism', characterised by severe necrotie dermatologic injuries (Swanson & Vetter 2006). One of the most well-known recluse species is the Mediterranean recluse spider, *L. rufescens* (Dufour, 1820). The original range of this species is in the circum-Mediterranean region, however it has been spread widely, most likely through accidental human transportation (Vetter 2008). It is now found in many temperate and tropical areas including the islands of the Atlantic, Madagascar, the Hawaiian islands, Australia, Mexico, and the United States (Gertsch & Ennik 1983). In Asia, *L. rufescens* has been reported in India (Tikader 1963), China (Chen & Gao 1990; Chen & Zhang 1991; Song et al. 1999), Russia (Dunin 1992), Taiwan (Song et al. 1999), Japan (Yaginuma 1940, 1986; Yoshikura 1987; Ono 2009), and South Korea (Namkung 2002). A recent molecular phylogenetic study of the *L. rufescens* complex suggested that *L. rufescens* might have occurred in Malaysia, although limited numbers of specimens were used for the study (only one female and two males) (Duncan et al. 2010).

Here, we report the finding of a large population of *L. rufescens* in a natural habitat in Southeast Asia. We doeument the presence of *L. rufescens* in western Thailand, provide observations on its morphology, natural history, habitat and identification, and propose how it may have arrived in Thailand.

METHODS

We surveyed six natural limestone caves located in the Khao Wang Khmer area of Kanchanaburi province in the western part of Thailand (14°25′N, 98°52′E) on 18 April and 25 May 2015 (Figs. 1, 2). These caves are located in the conservation area of the Plant Genetic Conservation Project under the Royal Initiative of Prineess Maha Chakri Sirindhorn (RSPG). Spiders were hand collected and specimens were preserved in 95% ethanol and transferred to the Center of Excellence in Entomology, Chulalongkorn University (Bangkok) for dissection and identification.

Spiders that appeared to be *L. rufescens* were examined to eonfirm their identity, and to provide morphological data on the population found in Thailand. The genitalia of females were dissected and cleared with a 3M KOH aqueous solution. An Olympus SZ60 stereoscope coupled with an Olympus digital camera (Camedia c-4040 zoom) was used to photograph the diagnostic features of the putative *L. rufescens*. All measurements (in millimeters) were carried out under an ocular micrometer in a stereomicroscope (Olympus: Zeiss Stemi DV4). Descriptions are based on one specimen for each sex; however, for the leg measurements (left legs), we also displayed measurements for an additional 5♀ and 4♂ speeimens (Table 1).

The following abbreviations are used throughout the text: AME, anterior median eye/s; CL, carapace length; CW, earapace width (measured at the widest point); ED, endite; LA, labium; LE, lateral eye/s; SL, sternum length; SW, sternum width (measured at the widest point); and TL, total length.

Specimens were identified in accordance with Greene et al. (2009). All voucher specimens (i.e., 10 ♀, 4 ♂ and 6 subadult juveniles) are deposited at the Chulalongkorn University Museum of Zoology (CUMZ), Bangkok (Thailand) for future analysis.

SYSTEMATICS

Family Sicariidae Keyserling, 1880
Genus *Loxosceles* Heineken & Lowe, 1832

Loxosceles Heineken & Lowe, 1832 in Lowe, 1832: 321. Full synonymy: see Gertsch & Ennik (1983) and Platnick (2015).

Figure 1.—The 'Death Railway' trail (black dashed line), which is in close proximity to Tum-Wangpra cave (red spot) where specimens of *Loxosceles rufescens* were found. Yellow spots are caves that we explored without finding any *L. rufescens*. The inset picture on the top right is indicative of what remains of the railway nowadays, whereas the inset picture on the bottom right shows the locality of Tum-Wangpra cave.

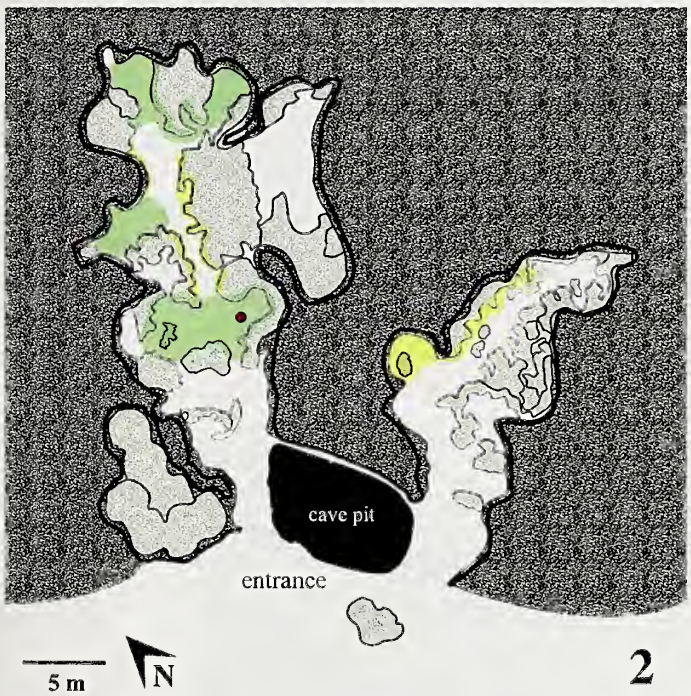


Figure 2.— A schematic outline of Tum-Wangpra cave located in the Khao Wang Khmer area of Kanchanaburi province, Thailand. Light green shading indicates areas where more than 100 *L. rufescens* individuals were found (residing mostly under scattered rocks on the ground), whereas yellow shading indicates areas with less than 100 individuals (and most specimens hiding in crevices on the cave walls). Dark grey areas depict the limestone cave boundaries, and light grey areas illustrate small limestone boulders that are accessible by humans. The red spot corresponds with the red arrow in Fig. 6, showing the exact location of the spider aggregation site.

Type Species.—*Scytodes rufescens* Dufour, 1820, by subsequent designation of Bonnet (1957).

Remarks.—As is typical of the genus *Loxosceles*, all specimens examined from Thailand possessed the following suite of characters: carapace flattened, longer than wide with a deep fovea (Fig. 5); clypeus porrect (Fig. 5); legs long and slender; sternum longer than wide; and abdomen oblong bearing spine-like setae (Lotz 2012). A diagnostic character for the females of *L. rufescens* is the structure of the spermathecae, which are characterized by the presence of closely-spaced receptacles and wide, laterally brown, sclerotized copulatory tubes (Fig. 3). These features were found in all 10 female specimens examined. In addition, when we examined four male specimens, the palpal tibia length to height ratio was less than 2.0, the cymbium was about half to less than half the length of the tibia, the length of the cymbium was similar to that of the pulpal bulb, and the palpal bulb was globular with a thin embolus (Fig. 4). These male characters are generally diagnostic of *L. rufescens* (Lotz 2012), although not exclusively so (Duncan et al. 2010; Planas & Ribera 2015; Planas et al. 2015).

Loxosceles rufescens (Dufour, 1820)
(Figs. 3–5, 8, 9)

Scytodes rufescens Dufour, 1820: 203, pl. 76, fig. 5. Full synonymy: see Platnick (2015).

Material examined.—THAILAND: *Kanchanaburi*: 6♀, 3 subadult juveniles, Sai Yok district, Tum-Wangpra cave, 14°24′47″N, 98°51′43″E, 18 April 2015, hand collected, N. Chomphuphuang (CUMZ-AR-ARA-Sic.2015.1–9); 4 ♂, 4♀,

Table 1.—Left leg and palp measurements of a single representative adult female and adult male of *Loxosceles rufescens* from Thailand, and averages and standard deviations of five additional females and four male specimens (all measurements are in millimeters). Leg formulas are provided.

	I	II	III	IV	Palp
Female (CUMZ-AR-ARA-Sic.2015.1); leg formula: 2413					
Femur	5.04	5.40	4.8	5.52	1.35
Patella	1.11	1.17	0.99	0.90	0.33
Tibia	5.28	6.56	4.80	5.46	0.87
Metatarsus	5.70	6.00	5.10	6.60	-
Tarsus	1.38	1.25	1.00	1.25	1.08
Total	18.51	20.38	16.69	19.73	3.63
Male (CUMZ-AR-ARA-Sic.2015.10); leg formula: 2413					
Femur	5.12	7.00	4.74	5.52	1.41
Patella	1.17	1.05	0.90	1.05	0.30
Tibia	6.80	8.40	5.90	5.92	0.90
Metatarsus	6.63	8.63	6.10	7.25	-
Tarsus	1.50	1.74	1.20	1.65	0.60
Total	21.22	26.82	18.84	21.39	3.21
Five adult females (CUMZ-AR-ARA-Sic.2015.2–6); leg formulas: 2143					
Femur	4.29±0.99	4.56±0.99	3.96±0.86	4.38±0.99	0.93±0.32
Patella	0.90±0.21	0.87±0.36	0.92±0.19	0.86±0.17	0.37±0.05
Tibia	3.72±1.85	4.23±2.21	3.14±1.51	3.59±1.75	0.53±0.22
Metatarsus	4.28±1.28	4.70±1.36	4.06±1.07	4.44±1.37	-
Tarsus	1.38±0.16	1.39±0.13	1.20±0.20	1.23±0.24	1.00±0.22
Total	15.24±3.54	16.52±3.96	13.78±3.07	15.13±3.29	2.65±0.42
Four adult males (CUMZ-AR-ARA-Sic.2015.10–13); leg formulas: 2143					
Femur	5.06±0.55	6.18±0.95	4.54±0.56	4.88±0.82	1.05±0.24
Patella	1.02±0.14	0.94±0.30	1.08±0.35	0.96±0.08	0.38±0.10
Tibia	6.15±0.86	7.40±1.16	4.85±0.87	5.28±0.76	0.55±0.26
Metatarsus	5.86±0.72	7.26±1.35	5.30±0.79	6.29±0.83	-
Tarsus	1.45±0.13	1.51±0.20	1.25±0.06	1.44±0.25	0.40±0.14
Total	14.51±8.78	17.11±10.84	12.91±7.97	14.05±8.60	1.95±1.21

3 subadult juveniles, same data except 25 May 2015 (CUMZ-AR-ARA-Sic.2015.10–20).

Description.—Carapace flattened, longer than wide, with a deep fovea and porrect clypeus (Fig. 5); chelicerae joined basally, with an immovable, thumb-like extension on the medial apical surface and short fangs; six eyes in three diads; legs long and slender, with two tarsal claws bearing serrated bristles on a small onychium; female genitalia haplogyne, with single broad opening and two spermathecae.

Female (CUMZ-AR-ARA-Sic.2015.1): TL = 8.1; CL = 3.6, CW = 3.2; eye diameter 0.15; AME-LE = 0.27; eye row strongly recurved; abdomen length = 4.26, width = 3.0; clypeus height = 0.32, SL = 1.88, SW = 1.59; LA = 0.78 long, 0.66 wide; ED = 1.29 long, 0.36 wide; leg formula: 2413; leg and palp measurements shown in Table 1. Carapace and chelicerae light brown, anterior carapace with dark brown 'violin' pattern (Fig. 5); legs and palps pale yellow to orange covered by short black setae, female palp without claw; coxae and sternum pale

Figures 3–4.—Diagnostic characters of *Loxosceles rufescens*: 3, female spermathecae (dorsal view); 4, male pedipalp. Scale bars = 0.15 mm (Fig. 3), 0.5 mm (Fig. 4).

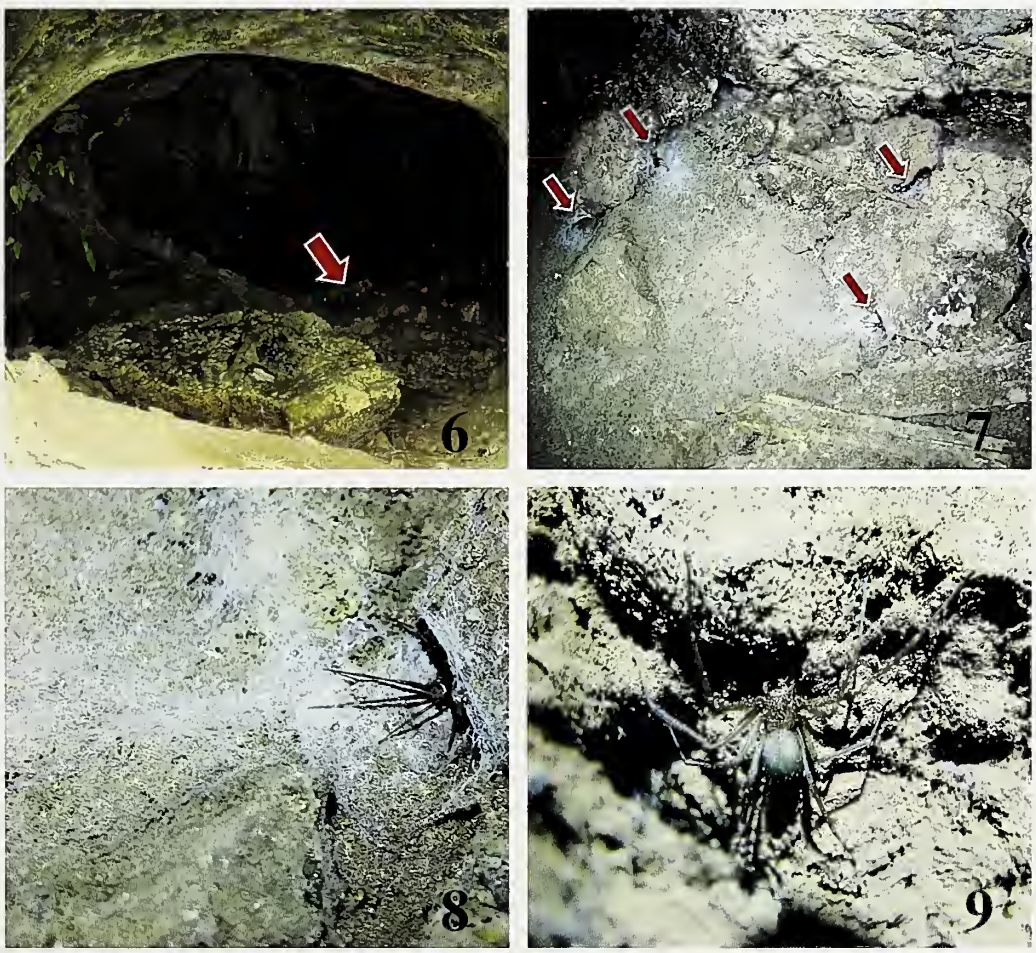
Figure 5.—Adult female *Loxosceles rufescens* carapace (dorsal view). Scale bar = 1 mm.

yellow, labium and endites brown; abdomen yellowish, covered with setae. Receptacles of spermathecae closely spaced; copulatory tubes wide with lateral brown sclerotized area (Fig. 3).

Female variation: A single female specimen (CUMZ-AR-ARA-Sic.2015.2) has two black spots on the posterior end of the carapace; subadults or recently molted individuals with pale pigment in the violin pattern.

Male (CUMZ-AR-ARA-Sic.2015.10): TL = 5.10; CL = 2.68, CW = 2.40; eye diameter 0.09; AME-LE = 0.21; eye row strongly recurved; abdomen length = 3.00, width = 1.55; clypeus = 0.27; SL = 1.23, SW = 1.35; LA = 0.45 long, 0.51 wide; ED = 0.90 long, 0.33 wide; leg formula: 2413 (see discussion); leg and palp measurements shown in Table 1. Overall body and coloration similar to female except for the abdomen, which is distinctly smaller than that of the female. Palpal tibia length to height ratio less than 2.0; cymbium half of tibia length; cymbium as long as length of palpal bulb; bulb globular and embolus thin (Fig. 4).

Eggs: In the laboratory, we retrieved a silken egg sac from one female nine days after the collection date. The sac contained 24 eggs with egg diameters between 1.07–1.15 mm. The eggs took 23 days after the egg sac was deposited to hatch



Figures 6–9.—Tum-Wangpra limestone cave, the locality where *Loxosceles rufescens* specimens were collected: 6, entrance to the cave (red arrow indicates the area where numerous *L. rufescens* were found); 7, cave surface (red arrows indicate *L. rufescens* retreats on the limestone ground); 8, flocculent web of *L. rufescens* on the crevice of the limestone ground; 9, *L. rufescens* clinging to the cave wall.

into spiderlings under an ambient temperature of 27°C and 70% relative humidity. Only 21 individuals successfully hatched (87% hatch rate). We measured the total body length of the spiderlings two days after they emerged from the egg sac, at which time they were between 1.20–1.68 mm (average 1.46 mm).

Habitat: Of the six limestone caves and peripheral urban areas that we surveyed, *L. rufescens* were found in only one of the caves locally known as "Tum-Wangpra" (Fig. 2). This cave has two major areas that extended approximately 20–25 m from the cave entranee. The accessible area for humans is about 340 m^2 with an average height of 5 m above ground. The cave is partially accessible by sunlight at a distance of 5 m from the cave entrance, which is devoid of the spiders. The surface of the ground inside the cave is slightly warm (annual average air temperature = 27°C and annual average relative humidity = 81.6 %), and mainly covered with a dry loamy sand and bat guano (Fig. 6). A population of the Old World hog-nosed bat, *Craseonycteris thonglongyai* Hill, 1974 is also found inhabiting the cave. An intensive survey revealed that the spiders were aggregated in similar microhabitats throughout the cave (Figs. 7–9); they hid themselves in crevices on the walls (Fig. 9), or under scattered rocks on the ground (Fig. 7–8) where they made small retreats of flocculent silk. Some individuals of *L. rufescens* also clung on rocks beneath other spiders' webs. By counting the visible spiders, we determined that there might be approximately 500 or more *L. rufescens* individuals living in the cave.

DISCUSSION

L. rufescens is a cosmopolitan species widely distributed throughout the world (Platnick 2015). Although native to the Mediterranean region, the species has been spread to other areas by human activities (Harvey 1996). In Israel, *L. rufescens* is moderately common in houses and basements (Shulov et al. 1962). Greene et al. (2009) suggested that populations of the genus *Loxosceles* in the United States tend to be extremely dense in favorable urban environments such as steam tunnels and subterranean habitats. In Iran, *L. rufescens* is also found inside buildings and under rocks and logs in urban areas (Zamani & Rafinejad 2014). In the natural environment, *Loxosceles* populations can be found in caves and/or cavern-like habitats (see Newlands 1975; Gertsch & Ennik 1983; Griffin 1998; Ferreira et al. 2005; Gonçalves-de-Andrade et al. 2007). Here, we report the anomalous discovery of *L. rufescens* in a natural habitat in Thailand. Until now, *L. rufescens* has never been reported in a natural habitat outside of its native range in the Mediterranean region. Clearly, the description of the physical parameters of the cave in which the spiders were found, and an understanding of their basic biology, are important considerations for determining the colonization requirements of this species in the target area.

Although speculative, the occurrence of *L. rufescens* in this part of Thailand might be explained by passive transportation by humans during World War II. The area in which we discovered *L. rufescens* was called the 'Hellfire Pass', when it was a major route for the construction of the infamous "Death Railway" or the Burma-Siam Railway along the Mae Klong River in Kanchanaburi province (Waterford 1994). The entrance of Tum-Wangpra cave is in close proximity to the railway (Fig. 1); material for the construction of the railway may have harbored the spiders, since specific railing material had to be shipped from Japan during that period, and there were already reports of *L. rufescens* present in Japan before 1940 (Bösenberg & Strand 1906; Strand 1918; Yaginuma 1940).

In North and South America, studies on the biology, distribution, and medical aspects of *Loxosceles* are ongoing, particularly for the brown recluse spider *L. reclusa* Gertsch & Mulaik, 1940, which is endemic to the USA (Swanson and Vetter 2005). In contrast, research on the genetic diversity and venom potency of *L. rufescens* has only been extensively studied in recent years (Planas & Ribera 2015; Planas et al. 2015). A protein expression analysis of the sphingomyelinase D (SMase D) protein, which is considered to be the major component of *Loxosceles* venom that causes dermatological injuries (Binford et al., 2008), suggested that the SMase D protein activity in *L. rufescens* venom is as high as in other *Loxosceles* species (Planas et al. 2015). In 2014, it was reported that a man in Phrae province in the northern part of Thailand had been bitten by *L. reclusa* and died (Bangkokpost 2014). The symptoms reported were very similar to loxoscelism, and this news sparked much attention and in some cases hysteria from the general public, much of it unwarranted. However, a doctor later concluded that the man died as a result of a secondary bacterial infection due to an unidentified spider's bite, and not loxoscelism as originally thought. This hypothesis agrees with our survey of the village perimeter near where the man was bitten, which did not yield any *L. reclusa*. Thus, in Thailand there is still no verified report of a human being bitten by a *Loxosceles* spider.

Our next goal is to survey the distribution of *L. rufescens* beyond the Khao Wang Khmer area to identify the true range of the species. Molecular studies of the Thai *L. rufescens* are equally important for determining the spider's origins. Indeed, since genetic diversity can be high within speeies of *Loxosceles* that share similar spermathecal and palp morphologies (Duncan et al. 2010; Planas & Ribera 2015; Planas et al. 2015), our samples need to be tested to determine whether they are genetically consistent with *L. rufescens* s. s.

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Chromosomal analyses of Salticinae and Lyssomaninae reveal a broad occurrence of the 2n♂ = 28, X_1X_20 karyotype within Salticidae

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Abstract. Brazil possesses the richest fauna of Salticidae in the world, including 560 species; however, no representative of the Brazilian fauna has been cytogenetically analyzed up to now. It has been demonstrated that karyotype data are a useful source for discussions on the phylogeny and chromosome differentiation of some salticid lineages. In this work, the first chromosome study of salticid species from Brazil is presented, with the addition of five genera to the 38 previously investigated worldwide. The analysis of mitotic and/or meiotic cells revealed 2n♂ = 28, X_1X_20 in *Asaracus* sp., *Coryphasia* sp., *Chira* sp., *Frigga quintensis* (Tullgren, 1905), and *Lyssomanes pauper* Mello-Leitão, 1945. This karyotype constitution is the most common for Salticidae, occurring in species of distinct clades. The diploid number 2n♀ = 28 observed in *Hasarius adansoni* (Audouin, 1826) is unexpected, differing in one autosomal pair from the karyotype previously registered for males of the same species. The cytogenetic information reported here reinforces the wide occurence of 2n♂ = 28, X_1X_20 within Salticidae, including species belonging to different clades and biogeographical regions. This karyotype is a shared character of Salticidae + Philodromidae, found exclusively in these families within Dionycha, suggesting its sister relationship already proposed in the literature.

Keywords: Jumping spider, meiosis, sex chromosome system, diploid number, chromosome evolution

A hundred years has past since Painter (1914) cytogenetically studied a salticid spider for the first time, *Maevia inclemens* (Walckenaer, 1837) [under *Maevia vittata* (Hentz, 1846)]. Despite the huge contribution of Maddison (1982, 1996) and Maddison & Leduc-Robert (2013), which cytogenetically analyzed 86 salticids, only 155 species belonging to 38 genera were karyotyped up to now (Araujo et al. 2016). This number corresponds to only 2.65% of the 5,850 taxonomically described Salticidae species (World Spider Catalog 2016). Furthermore, many clades, mainly those predominantly composed of Neotropical speeies (Amycoida, Marpissoida, Euophryini and Freyina) or basal species (non-salticines) (Maddison 2015) remain almost unknown from the karyological point of view. Only six Neotropical Salticidae species, belonging to the genera *Bryantella* Chickering, 1946, *Dendryphantes* C.L. Koch, 1837, *Metaphidippus* F.O.Pickard-Cambridge, 1901 (Salticinae, Dendryphantini, Dendryphantina) (Scioseia 1997) and *Habronattus* F.O.Pickard-Cambridge, 1901 (Salticinae, Plexippini, Harmochirina) (Maddison & Leduc-Robert 2013) were cytogenetically studied, and, among these genera, only *Bryantella* is exclusively Neotropical (World Spider Catalog 2016). Within non-salticines, only *Holcolaetis vellerea* Simon, 1910 (under *Holcolaetis vidua* Lessert, 1927) (Spartaeinae, Spartaeini, Holcolaetina) was karyotyped (Mittal 1961, 1964).

At a first glance, the salticids cytogenetically analyzed seem to constitute a relatively homogeneous group, mostly composed of species with 2n♂ = 28, X_1X_20 (Araujo et al. 2016). A multiple sex chromosome system (SCS) of the X_1X_20 type is rare in other animal groups but the most common in spiders (see Araujo et al. 2012). In this SCS, the sex is not determinate by the presence of an Y or W chromosome, as it occurs in most mammals and birds. The number of copies of each X chromosome determines the sex, a single copy of each one (X_1X_20) is characteristic of a male and two copies of each one ($X_1X_1X_2X_2$), characterizes a female. The "0" after the X_1X_2 denotes the absence of a Y chromosome in male complement. Thus, in this SCS, if the male diploid number is 2n♂ = 28 (26 autosomes plus X_1X_2), the female diploid number is 2n♀ = 30 (26 autosomes plus $X_1X_1X_2X_2$). At the end of male meiosis I, both X_1 and X_2 segregate to the same cell pole and the opposite pole contains no sex chromosomes. Recently, a study showed that uncommon karyotypes in spiders, with a multiple sex chromosome system including a Y chromosome (X_1X_2Y and $X_1X_2X_3Y$), occur in *Habronattus*, and probably evolved independently several times within this genus (Maddison & Leduc-Robert 2013).

Cytogenetic studies on underrepresented salticid clades could provide us with information about the homogeneity or heterogeneity of some lineages and could be useful for discussions concerning salticid systematics. Thus, the goal of this study is to analyze the chromosomes of six Salticidae species from Brazil: the Salticinae *Asaracus* sp., *Chira* sp. and *Frigga quintensis* (Tullgren, 1905) (Aelurillini), *Coryphasia* sp. (Euophryini), and *Hasarius adansoni* (Audouin, 1826) (Hasar-

Table 1.—Salticid spiders investigated in this work with their respective samples and collection localities in Brazil. SP = state of São Paulo, MS = state of Mato Grosso do Sul, PR = state of Paraná. Classification according to Maddison (2015).

Taxa	Sample	Collection locality
Salticinae, Saltafresia, Aelurillini, Freyina		
Asaracus sp.	1♂	Margem da Lagoa Xambrê, Parque Nacional de Ilha Grande, Altônia (23°52′21″S, 54°00′01″W), PR
Chira sp.	1♂	Rio Claro (22°24′00″S, 47°34′19″W), SP
Frigga quintensis (Tullgren, 1905)	2♂	Margem da Lagoa Xambrê, Parque Nacional de Ilha Grande, Altônia (23°52′21″S, 54°00′01″W), PR; Ivinhema (22°18′00″S, 53°49′16″W), MS
Salticinae, Saltafresia, Euophryini		
Coryphasia sp.	1♂	Rio Claro (22°24′00″S, 47°34′19″W), SP
Salticinae, Saltafresia, Hasariini		
Hasarius adansoni (Audouin, 1826)	1♀	Ivinhema (22°18′00″S, 53°49′16″W), MS
Lyssomaninae		
Lyssomanes pauper Mello-Leitão, 1945	4♂,3♀	Reserva Particular do Patrimônio Natural da UFMS (20°29′58″S, 54°36′48″W), Campo Grande, MS

iini), and the Lyssomaninae *Lyssomanes pauper* Mello-Leitão, 1945.

Except for the genus *Hasarius* Simon, 1871, karyotyped by Suzuki (1951, 1954), the remaining genera were not previously cytogenetically analyzed (Araujo et al. 2016).

METHODS

The number of individuals and collection localities of the species examined in this work are listed in Table 1. Collecting permits were provided by the Instituto Brasileiro do Meio Ambiente e dos Recursos Renováveis – IBAMA and Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio (15382-1 and 15157-1). The voucher specimens were deposited in the arachnologieal eollection of the Laboratório Especial de Coleções Zoológicas, Instituto Butantan (IBSP, curator A. D. Brescovit), São Paulo, state of São Paulo, Brazil. The chromosome preparations were obtained following Araujo et al. (2008), i.e., the gonads were submitted to 2 hours in a treatment with 0.16% colchicine solution (diluted on physiologic solution: 7.5 g NaCl, 2.38 g Na_2HPO_4, 2.72 g KH_2PO_4, in 1 l of distilled water), 15 minutes in a hypotonic treatment with tap water, and fixation with methanol/acetic acid (3:1). Later, the gonads were dissociated in 45% acid acetie solution on the surface of a microscope slide that was heated to 35 °C/ 40 °C, and standard stained with 3% Giemsa solution (3% of commercial Giemsa and 3% of phosphate buffer pH 6.8 in distilled water) for 12 min. At least 30 cells were considered in the analysis of each species. The chromosome morphology was determined following the nomenclature proposed by Levan et al. (1964). Difficulties in obtaining certain stages of cell division occurred due to the low number of specimens, for most species, or the development stage of the individuals, as in the case of *L. pauper*.

RESULTS

Male diplotene/metaphase I cells of *Asaracus* sp., *Coryphasia* sp., *Chira* sp. and *F. quintensis* are composed of 13 autosomal bivalents and two sex univalents (X_1 and X_2). Thus, the meiotic formula of these species is $13II+X_1X_20$. The number of chiasmata per bivalent is usually one, localized on terminal, interstitial or proximal regions, but some bivalents with two terminal ehiasmata can be observed in some cells. The sex chromosomes can be easily identified due to their positive heteropycnosis and/or peculiar disposition, since they normally appear side by side or at least close to each other (Fig. 1A–D). The positive heteropycnosis of the sex chromosomes is detected even at pachytene, in which it is possible to observe the X_1 and X_2 closely packed, being difficult to establish their limits (Fig. 1E), or clearly separated from each other (Fig. 1F). Male metaphase II cells of *Asaracus* sp. and *F. quintensis* exhibited n = 13 or n = 15, confirming the regular segregation of the X_1 and X_2 chromosomes to the same pole at anaphase I (Fig. 1G, H). In some metaphase II nuclei, the sex chromosomes cannot be distinguished from the autosomes (Fig. 1G), but in others, these elements presented a positive heteropyenosis (Fig. 1H).

Spermatogonial prometaphase/metaphase cells of *Coryphasia* sp., *F. quintensis* and *L. pauper* showed 2n♂ = 28, X_1X_20 (Fig. 1I, K), which is compatible with the meiotic findings and allow us to conclude that all salticid species above mentioned possesses 2n = 28, X_1X_20 in males and 2n = 30, $X_1X_1X_2X_2$ in females. Unfortunately, only female specimens of *H. adansoni* were collected. The analysis of oogonial metaphases of *L. pauper* and *H. adansoni* revealed the diploid number of 2n = 30 and 2n = 28, respectively (Fig. 1L, M). The chromosomal morphology was not established due to the irregular shape of the elements and/or absence of mitotic plates, except for the chromosomes of *L. pauper* and *H. adansoni*, which are clearly telocentrics.

DISCUSSION

The data presented in this work are the first for salticid species collected in Brazil. This seems to be fundamental considering that Maddison & Hedin (2003) and Maddison et al. (2008) suggest a biogeographical division between Old World and New World salticid fauna. In addition, almost all new world salticids cytogenetically analyzed are from North America (Pinter & Walters 1971; Maddison 1982, 1996; Tugmon et al. 1990; Maddison & Leduc-Robert 2013), despite the fact that Brazil possesses the richest salticid fauna in the world, with 560 species (Metzner 2016). All Salticidae species analyzed here, except *H. adansoni*, revealed meiotic and/or mitotic features that are consistent with 2n♂ = 28, X_1X_20, the

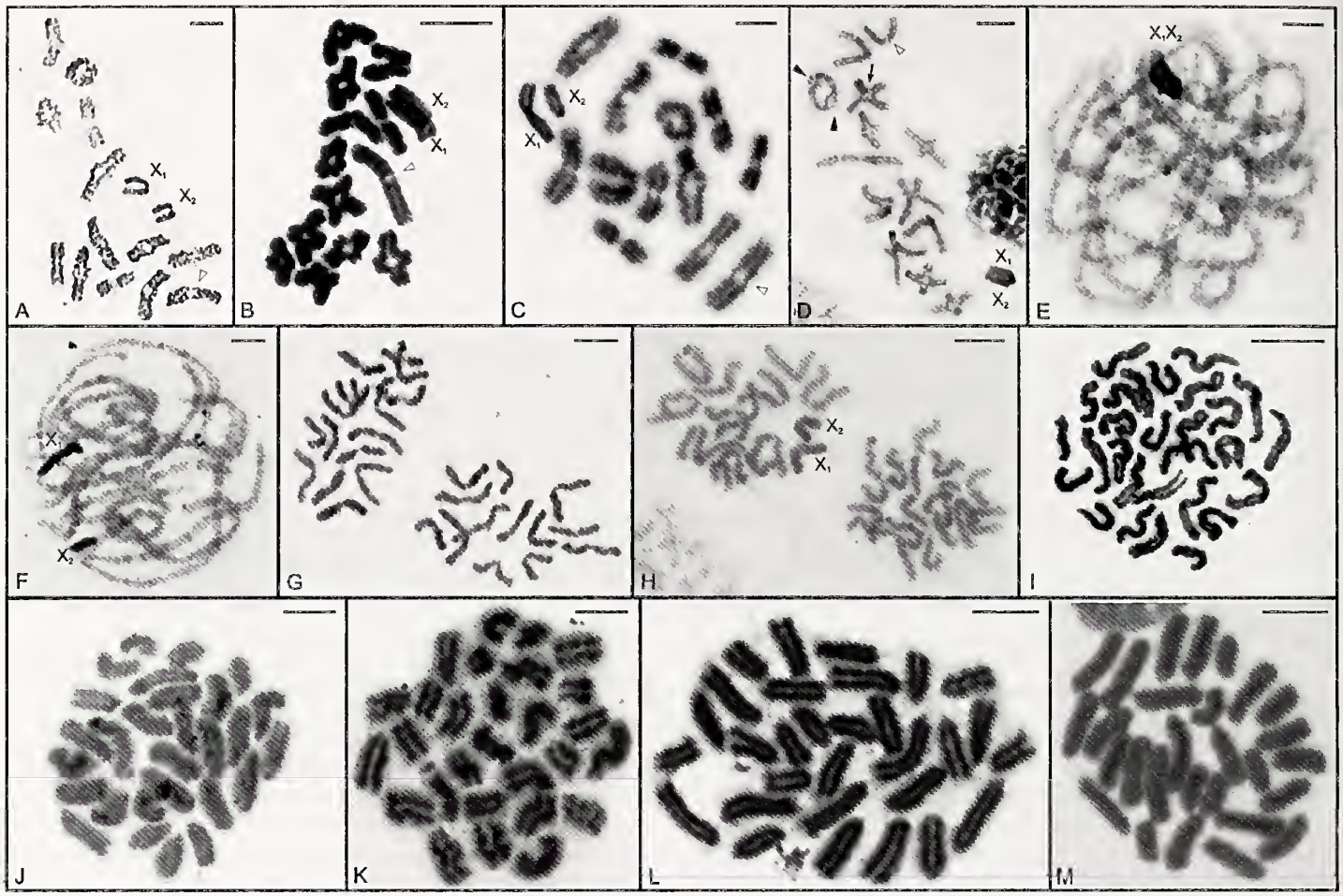

Figure 1.—Meiotic and mitotic cells of the salticid species. A–D. Diplotene/metaphase I spermatocytes of *Asaracus* sp. (A), *Coryphasia* sp. (B), *Chira* sp. (C) and *Frigga quintensis* (D) with 13 autosomal bivalents plus two sex univalents (X_1 and X_2). For exemplification, the arrow indicates one autosomal bivalents with one interstitial chiasma, the empty arrowheads show autosomal bivalents with one terminal chiasma, and the full arrowheads point to two terminal chiasmata in one autosomal bivalent. E, F. Pachytene cells of *Chira* sp., showing the positive heteropycnosis of the sex chromosomes, that appear together (E) or separated (F). G, H. Metaphase II cells of *Asaracus* sp. (G) and *Frigga quintensis* (H) with n = 13 in the right and $n = 13 + X_1X_2$ in the left. In (H) it is possible to identify the positive heteropycnotic sex chromosomes. I–K. Spermatogonial prometaphase/metaphase cells of *Coryphasia* sp. (I), *Frigga quintensis* (J), and *Lyssomanes pauper* (K), with $2n♂ = 28$. L, M. Oogonial metaphase cells of *Lyssomanes pauper* (L), with $2n♀ = 30$ and *Hasarius adansoni* (M), with $2n♀ = 28$. Scale = 10 μm.

most common chromosome constitution observed in the family (Araujo et al. 2016).

Despite the fact that no quantitative analysis of chiasmata was carried out, the species analyzed here seem to show a tendency to have only one chiasma per bivalent, a common characteristic in spiders (White 1973). However, in contrast to the observations of White (1973) suggesting that the chiasmata are primarily proximal in spiders, the nuclei of the species analyzed herein show a diversity of chiasma position (distal, interstitial and proximal). This pattern was already described for *Habronattus* species with a X_1X_20 system, contrasting to the Y-possessing species of the same genus, which showed a tendency to have distal chiasmata (Maddison & Leduc-Robert 2013). Chiasmata in other positions than distal could act against the occurrence of chromosome fusions that form metacentric elements, including metacentric neo-Ys (for a more detailed discussion see White 1973 and Maddison & Leduc-Robert 2013).

Based on our results, *Asaracus*, *Chira* and *Frigga* ($2n♂ = 28$, X_1X_20) are the only genera, along with *Aelurillus* Simon, 1884, with described karyotypes within the tribe Aelurillini (Maddison 2015).The karyotype data of *Aelurillus politiventris* (O. P-Cambridge, 1872), from Israel, is $2n♂ = 21$, X0 (Gorlova et al. 1997). This difference in diploid number and sex chromosome system can reflect the biogeographical distribution of these genera. Instead of belonging to the same tribe Aelurillini, the first three genera are part of the Neotropical subtribe Freyina, and *Aelurillus* belongs to the Afro-Eurasian subtribe Aelurillina (Maddison 2015). Thus, within Aelurillini, the karyotype seems to be subtribe-specific, but it is important to emphasize that most genera, including the entire African subtribe Thiratoscirtina, are cytogenetically unknown.

Euophryini, which encompasses *Coryphasia* (Maddison 2015), possesses two other species that were cytogenetically studied: *Euophrys pseudogambosa* Strand, 1915, from Israel (Gorlova et al. 1997) and *Jotus minutus* L. Koch, 1881 (Suzuki 1951), both also with $2n♂ = 28$, X_1X_20. In the phylogenetic

hypothesis of Zhang & Maddison (2015), *Coryphasia*, *Euophrys* C.L. Koch, 1834 and *Jotus* L. Koch, 1881 belong to distinct clades within Euophryinae (currently Euophryini, see Maddison 2015). However, despite the phylogenetic and geographical distance, all three genera share the same diploid number and sex chromosome system. *Neon* Simon, 1876, classically placed in Euophryinae (Prószyński 2012; Metzner 2016), was relocated to a new group, Astioida, by Maddison et al. (2008, 2014), which possesses only one cytogenetically analyzed genus, *Myrmarachne* MacLeay, 1839. A close relationship between *Neon* (Astioida, Neonini) and *Myrmarachne* (Astioida, Myrmarachnini) is proposed by the cytogenetic data, because *Neon reticulatus* (Blackwall, 1853), from Turkey, exhibits 2n♂ = 21, X0 (Kumbıçak 2014) and four of the six *Myrmarachne* species analyzed cytogenetically showed 2n♂ = 23, X0 (Hackman 1948; Bole-Gowda 1958); these karyotypes are much more similar to each other than to the 2n♂ = 28, X_1X_20 found in the Euophryini *Coryphasia*, *Euophrys* and *Jotus*.

Some considerations should be addressed regarding the female specimen of *H. adansoni* (Hasariini) with the unexpected 2n♀ = 28. . Taking into account that Suzuki (1954) described 2n♂ = 28, X_1X_20 in *H. adansoni*, it was supposed that females of this species would have a diploid number of 2n♀ = 30, $X_1X_1X_2X_2$. However, this female specimen has a diploid number lower than the expected. Even though the sex chromosome system was not identified in the present work, if we consider the same system found by Suzuki (1954), the female specimen analyzed here probably has the constitution 2n♀ = 28, $X_1X_1X_2X_2$. Thus, this could be a case of polymorphism in the number of autosomes, that is, 26 autosomes in the Japanese population studied by Suzuki (1954) and 24 autosomes in the population from Ivinhema, state of Mato Grosso do Sul, Brazil (present work). Polymorphism in the number of autosomes was already described in another salticid species, *Habronattus viridipes* (Hentz, 1846), in which Maddison (1982) found 2n♂ = 28, X_1X_20 in most males, and 2n♂ = 30, X_1X_20 in only one specimen. Both cases, show a discrepant chromosome number in only one specimen. Nevertheless, the presence of this unusual diploid number within the *H. adansoni* population from Ivinhema, Brazil, needs still to be confirmed. Without a more thorough collection effort, a distinction between a population polymorphism (Japan x Brazil) or an individual chromosome variation eannot be made. In fact, the 2n♂ = 28, X_1X_20 observed in other eytogenetically analyzed Hasariini, *Habrocestum rubroclypeatum* Lessert, 1927 (Mittal, 1964), reinforces the hypothesis that the 2n♀ = 28 encountered in the female specimen of *H. adansoni* from Ivinhema is a populational variation of the karyotype 2n♂ = 28/2n♀ = 30, which is commonly described for Salticidae.

Lyssomanes pauper is the first Lyssomaninae karyotyped, but the occurrence of 2n♂ = 28, X_1X_20 in this species and the other non-salticine cytogenetically studied, *Holcolaetis vellerea* (Mittal 1961, 1964) (Spartaeinae, Spartaeini), both basal salticids, points to a broad distribution of this karyotype within salticids. Moreover, the presence of 2n♂ = 28, X_1X_20 only known for Salticidae and Philodromidae within Dionyeha (see Araujo et al. 2016), suggests a sister relationship between these families, as already proposed by Ramírez (2014).

The data presented here reinforce the karyotypic homogeneity within salticids, with the 2n♂ = 28, X_1X_20 widespread in species of several clades and biogeographical regions. However, in many clades, only one or a few species were karyotyped so far, a situation that blurs an unrevealed chromosome heterogeneity, as shown for *Habronattus*, a genus that has around 66% of its 99 species already karyotyped (Araujo et al. 2016; World Spider Catalog 2016). This genus exhibited four different diploid complements, including the X_1X_2Y, $X_1X_2X_3Y$ and X0 sex chromosome systems, besides the classical 2n♂ = 28, X_1X_20 (Maddison & Leduc-Robert 2013).

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The hemolymph vascular system in *Araneus diadematus* with special focus on intraspecific variability in artery systems

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Abstract. The European garden spider, *Araneus diadematus* Clerck, 1757, is one of the most common spiders in central Europe. However, despite its abundance, comparatively little is known about its internal anatomy. We therefore conducted an examination of the hemolymph vascular system (HVS) of *A. diadematus*, as part of a comparative survey on the circulatory system in spiders. The HVS of *A. diadematus* was investigated using micro-computed-tomography and serial sectioning and visualized using 3D-reconstruction software. In order to examine the HVS for intraspecific variability, over 30 specimens were studied in detail. The HVS of *A. diadematus* consists of a tubular heart, which is situated along the dorsal midline of the opisthosoma. Anteriorly, the heart gives rise to the anterior aorta and posteriorly to the posterior aorta. Three pairs of cardiac arteries originate from the dorso-lateral section of the heart and the branching pattern of these arteries in *A. diadematus* is visualized and described here for the first time. The anterior aorta runs through the pedicel into the prosoma where it branches to supply the muscles and organs with hemolymph. The central nervous system in particular is supplied by a large number of arteries, which show some interesting branching patterns, e.g., a unilateral origin of the transganglionic arteries in the subesophageal ganglion, and the supply of the lower lip by the first of these transganglionic arteries. Furthermore, there are a number of arteries in the HVS with unilateral (asymmetrical) origins. Some cases of intraspecific variability are demonstrated, e.g., for the arteries of the upper and the lower lip. The data presented here are discussed in reference to information on the HVS in Araneae available in existing literature.

Keywords: 3D imaging, micro-CT, right-left asymmetry

The earliest descriptions of the hemolymph vascular system (HVS) in Araneae can be traced back to the beginning of the 19th century (e.g., Cuvier 1810, pp. 257–263; Treviranus 1812) and, in the decades which followed, there was a marked increase in the interest this organ system generated (Brandt 1840; Grube 1842; Blanchard 1849; Schimkewitsch 1884). Although there has been a lot of interest in the opisthosomal part of this organ system, the prosomal HVS has received less attention (Claparède 1863; Causard 1896; Petrunkevitch 1911; Willem 1917; Crome 1952). However, the particularly detailed results presented by Schneider (1892) and Causard (1896) have had a significant influence on our perception of the vascular system in spiders.

In essence, the circulatory system of Araneae, as in other arthropods, can be divided into two parts: a) the HVS, consisting of the heart and arteries and, b) the hemolymph lacunar system, comprising sinuses and lacunae (Wirkner 2009; Wirkner et al. 2013). The hemolymph is distributed throughout the body by the pumping heart via artery systems. At the ends of the arteries, the hemolymph leaves the HVS and enters the hemolymph lacunar system. Within this latter system, the hemolymph is channeled to the book lungs and from there the oxygenated hemolymph is channeled – via sinuses – to the pericardial sinus. Here the hemolymph reenters the heart lumen via ostia, slit-like openings in the wall of the heart (see e.g., Wirkner & Huckstorf 2013).

In the context of a comprehensive survey on the circulatory system in Araneae, we present here the results of a detailed study on the HVS in the species *Araneus diadematus* Clerck, 1757 using up-to-date morphologieal analysis and visualization methods. Morphological descriptions are usually generalized by species and, after a series of intermediate steps, it is possible to attain an evolutionary analysis of organ systems (Richter & Wirkner 2014). However the problem of intraspecific variability has often been neglected in morphological studies. In Crustacea, it is known that different arterial patterns can occur within one species (Keiler et al. 2013) and even within genetically homogenous populations as in *Procambarus fallax* (Hagen, 1870) f. *virginalis* (Vogt et al. 2009). To be able to effectively generalize morphological results with respect to one species, a large data set must be obtained. We studied 33 specimens of *A. diadematus* using micro-computed-tomography (micro-CT) and 3D-reconstructions to provide a detailed morphological description of the HVS, with special focus on intraspecific variability of arterial patterns.

METHODS

Animals.—The specimens of *A. diadematus* were collected in the urban area of Rostock (Mecklenburg-Western Pomerania, Germany) in October 2010, and between August and November 2011. Around 100 adult and penultimate females and seven adult males were obtained. The body length of the females ranged from 8–18 mm; in the males the range was between 5 and 6 mm. Only males were used for semi-thin sectioning (see below). Additionally, one specimen of *Araneus quadratus* Clerck, 1757 was studied. This sample was collected in September 2010 on the island of Hiddensee (Mecklenburg-Western Pomerania, Germany).

The spider taxonomy employed follows 'The World Spider Catalog' (2015).

Ninety-two of the female specimens were treated using the injection method – as detailed in the following section. To verify the quality of the injection, injected specimens were checked using micro-CT. Ultimately, 33 specimens (injected and uninjected) were scanned and used for further 3D investigation. Table 1 shows the different procedures used to

Table 1.—The applied treatments and methods for the considered 33 specimens. Ad: *Araneus diadematus*; Aq: *Araneus quadratus*; CPD: critical point dryer; KOH: potassium hydroxide.

Specimen-ID	Method	Sex	Injection (resin)	Tissue treatment	Contrasting treatment
Ad01, Ad03 Ad12, Ad13 Ad14, Ad15 Ad16, Ad17	micro-CT	f	PU4ii	KOH (maceration)	Lugol's solution
Ad04, Ad05 Ad07, Ad10 Ad11	micro-CT	f	-	Dubosq-Brasil	alcoholic Iodine-solution
Ad06	micro-CT	m	-	Dubosq-Brasil	alcoholic Iodine-solution
Ad08	micro-CT	f (penultimate)	-	Dubosq-Brasil	alcoholic Iodine-solution
Ad30	semi-thin sections	m	-	Dubosq-Brasil	azure II and methylene blue
Ad39, Ad40	micro-CT	m	-	Dubosq-Brasil	alcoholic Iodine-solution
Ad41, Ad43 Ad44, Ad45 Ad46, Ad47 Ad48	micro-CT	f	PU4ii	Bouin	PU4ii mixed with 1% iodine enriched Butanon; Lugol's solution
Ad69	micro-CT	f	PU4ii	Bouin	Lugol's solution; alcoholic Iodine-solution
Ad72, Ad86	micro-CT	f	PU4ii	Bouin	Lugol's solution
Ad92, Ad94	micro-CT	f	Mercox II	Bouin	alcoholic Iodine-solution; CPD
Ad95	micro-CT	f	Mercox II	Bouin	alcoholic Iodine-solution
Ad105	micro-CT	f	Mercox II	Dubosq-Brasil	alcoholic Iodine-solution
Aq01	micro-CT	f	PU4ii	Bouin	alcoholic Iodine-solution

treat eaeh of the 33 specimens. When referring to a specific specimen, the assigned Specimen-ID (see Table 1) is given, e.g., in figure legends.

Injection of resin.—To prepare casts of HVS, we followed the methods as described by Wirkner & Richter (2004). Spiders were anesthetized with a lethal dose of chloroform and then injected with resin. We used the casting resin Mercox II (Ladd Researeh Industries, USA) or the polyurethane Pu4ii (vasQtec, Univ. of Zurich, Switzerland). Just before being injected, the resin was mixed with a hardener (Pu4ii) or a catalyst (Mercox II) and put into a 5 ml syringe (Braun Injekt®, Luer Solo) that was locked onto a medical infusion set, 0.4 × 20 mm in size (servoprax, Wing Flo®, Luer Lock). The injection setup was finely adjusted using a mechanical mieromanipulator. The legs of the spiders were removed during the injection procedure so the resin could leave the body. To allow the resins to polymerize and temper, the specimens were left for several minutes after the injection. Mercox II-injected and uninjected specimens were fixed in Duboscq-Brasil fixative (10 parts: saturated picric acid dissolved in 96% ethanol, 4 parts: 37% formaldehyde, 1 part: glacial acetic acid), while Pu4ii-injected specimens were fixed in Bouin's fluid (15 parts: saturated aqueous picric acid, 5 parts: 40% formaldehyde, 1 part: acetic acid). To ensure enhanced contrast, preparations later used for miero-CT were stored for at least 12 hours in alcoholic iodine-solution or in Lugol's solution (aqueous iodine potassium iodide solution) (Metscher 2009). The prosomata and the opisthosomata of the animals were separated at the pedicel to aid the fixing and contrasting process. This method was exclusively applied to female specimens.

Specimens with a good injection result were eritical-point-dried (EMITECH K850, UK) to further ameliorate contrast.

Micro-computed-tomography (micro-CT).—X-ray scans were performed using the Phoenix Nanotom® (Phoenix|X-ray, GE Sensing & Inspection Technologies) high-resolution micro-CT system in high-resolution mode (target: molybdenum, mode: 0–1; performance: ca. 3–6 W; number of images: 1440; detector-timing: 1500–5000 ms; voxel size: ca. 4–8 µm). The surrounding medium of the speeimens during the sean was distilled water, ethanol, or air depending on the initial fixing and contrasting procedure.

Semi-thin section series.—Histological semi-thin section series were carried out for male specimens. For this purpose, the prosoma was removed from the opisthosoma and fixed in Bouin's fixative. It was then dehydrated in ethanol and, after an intermediate step of epoxypropane, embedded in araldite epoxy resin under vacuum. Serial semi-thin sections (1 µm) were made with a Leica Ultracut UCT microtome using a diamond knife. The sections were stained with a mixture of 1% azure II and 1% methylene blue in aqueous 1% borax solution for approximately 5–25 s at 80–90°C.

3D reconstruction.—The image stacks produced using micro-CT were loaded into the software Imaris 7.0.0 by Bitplane to create all 3D-reconstructions. A scene was created in the program module 'Surpass'. To visualize whole data sets, the volume rendering function was chosen. The contours of the studied morphological structures were marked with a polygon on virtual cross sections.

Images/Illustration.—All figure plates were arranged using the Corel Graphics Suite X3. Images were embedded into Corel Draw X3 files and edited in Corel PhotoPaint X3 using general imaging enhancement tools (contrast, brightness).

The PDF version of the current study is equipped with interactive 3D-content. Three-dimensional objects resulting from the aforementioned 3D-reconstruction process were converted using Adobe 3D-Reviewer (version: 9.0.0.107). To activate the 3D-content, click on the desired image and use the mouse buttons to navigate.

RESULTS

The following section predominantly comprises a description of the HVS in *A. diadematus* and, as the heart constitutes the central organ of the whole system, it is a logical starting point. The terminology employed follows the ontology of arthropod circulatory systems (OArCS; Wirkner unpubl. data). In accordance with this ontology, only single un-

branched arteries are termed 'artery system', such as 'first cardiac artery system'. Complete vascular trees, i.e., arteries and all their branches are termed 'artery system', for example 'first cardiac artery system'. An artery system is named after the stem artery, to which all its branches can be traced back.

The morphological descriptions that follow are divided into two parts. The first part comprises the description of stable morphological patterns, which were observed in all specimens. The second part, 'Variability', eonsiders morphological patterns that were found to differ between specimens. The numerical ratios by which eertain patterns were observed are given in this context.

HVS of the opisthosoma.—*The heart:* The heart of *A. diadematus* lies in the dorso-medial region of the opisthosoma. It runs in an anterior direction from the third pair of the dorso-ventral muscles to the posterior end of the pedieel. In the anterior part of the opisthosoma, the heart bends (in a slight s-shape) ventrally toward the pedicel but not directly adjacent to the anterior cuticle (the opisthosoma overhangs the pedicel here) (Figs. 1A & C, 2A).

At its posterior end, the heart merges into the posterior aorta. The posterior aorta runs towards the spinnerets where it splits at their distal end. The posterior aorta system does not show any further ramifications (Fig. 1B, D, E). Anteriorly, the heart is extended by the anterior aorta, which supplies the whole prosoma.

Three pairs of ostia are located on the lateral side of the heart and three pairs of cardiac arteries originate on the ventro-lateral side of the heart (Fig. 2). The first pair of ostia is situated on a dilatation of the myocard which is situated dorsally to the bending of the heart toward the pedicel. These ostia are oriented in a dorso-ventral direction. The second and the third pairs of ostia are located in the dorso-lateral region of the heart. They lie dorsally to the origins of the first and second cardiac artery systems, respectively. The second pair of ostia is located in the heart region between the most anterior pair of dorsal muscles and the first pair of dorso-ventral muscles. The third pair of ostia lies between the first and second pair of dorso-ventral museles. The third pair of cardiac artery systems arises laterally to the origin of the posterior aorta system at the posterior end of the heart.

Cardiac artery systems: Three pairs of cardiac artery systems originate from the heart. The first and second pairs of cardiac artery systems originate ventro-laterally to the second and third pair of ostia, respectively (Fig. 2A). The third pair of cardiac artery systems originates at the posterior end of the heart, laterally to the origin of the posterior aorta system. All three pairs of cardiac artery systems run to the periphery of the opisthosoma (Fig. 1B, interactive 3D content).

The first pair of cardiac artery systems mainly supplies the antero-lateral part of the opisthosoma (Fig. 1B, C). The seeond system supplies the central region of the opisthosoma (Fig. 2B, C) and it includes a prominent stem artery, the second cardiae artery, which runs to the ventral muscle cord. Numerous smaller arteries with anterior or posterior orientation originate from the second cardiac artery, which also divides into two equally sized arteries dorsally to the ventral muscle cord.

The third pair of cardiac artery systems supplies the postero-lateral part of the opisthosoma (Fig. 1B, C).

Variability: There is one artery of the third cardiac artery system (Fig. 1D, E; ca3*) that splits off medially and runs postero-ventrally to the stercoral pocket. Every specimen displayed a unilateral occurrence of this artery, however in six specimens the origin was observed to be at the right third cardiac artery, and at the left cardiac artery in a single specimen.

It should be noted that here we describe variable patterns of major arteries. Variability in smaller arteries is not in the scope of this study and should be a topic of further investigations (see also Discussion).

Ligaments of the heart: The heart is suspended by ten pairs of dorsal ligaments, nine pairs of lateral ligaments and three pairs of ventral ligaments (Fig. 2). The dorsal ligaments are attached to the dorsal midline of the heart at regular intervals. They are attached almost rectangular to the heart from where they run to the dorsal cuticle of the opisthosoma. Only the tenth dorsal ligaments run at a more oblique angle: in this case the dorsal attachment points are in a posterior direction. Eaeh pair of dorsal ligaments comprises two single ligaments, which lie close to each other at the attachment sites at the heart and diverge dorsally.

The lateral ligaments originate at the dorso-lateral side of the heart and run laterally to the cuticle. The third, seventh and ninth lateral ligaments attach at the apodemes of the dorsal muscle (dm), the second dorso-ventral muscle (dvm2) and third dorso-ventral muscle (dvm3), respectively (Fig. 2A, B).

The ventral ligaments originate at the ventro-lateral sides of the heart. The first pair of ligaments runs postero-ventrally between the dorsal muscles. The second pair of ventral ligaments originates medially to the origins of the second cardiac artery system and runs ventrally between the ovaries. The third pair of ventral ligaments originates medially to the third cardiac artery system and runs ventrally along the ventral side of the stercoral pocket (Fig. 2A).

HVS of the prosoma.—*Anterior aorta system:* The anterior aorta system supplies the whole prosoma (Fig. 3). The anterior aorta (Fig. 3B; aa) emanates anteriorly from the heart, which runs dorsally through the pedicel. On reaching the stomach, the anterior aorta splits into two aortic arches which flank the stomach laterally (Fig. 3B; interactive 3D content; aoa). Shortly after this first split, the paired third dorsal prosomal artery system (Fig. 3A, B; dpa3) splits off dorsally and supplies the dorsal musculature of the posterior prosoma by numerous ramifications. Anterior to the third dorsal prosomal artery system, the peribuccal artery system (Fig. 3B; pba) originates. This artery system supplies the dorsal dilatator musculature of the sucking stomach.

Posterior to the supraesophageal ganglion, the aortic arches bend ventrally and run along the dorsal surface of the subesophageal ganglion. In this region, the aortic arches give rise to four pairs of leg arteries (l1, l2, l3, l4), an unpaired first median transganglionic artery (mtga1) and an unpaired supraneural artery (sa). The pedipalpal arteries (pa) originate from the first leg arteries in close proximity to where they split off the aortic arches (Fig. 3A; interactive 3D content).

Cheliceral artery systems: The cheliceral artery systems (Fig. 3A, B; cha) originate in the dorsal bend of the aortic arches.

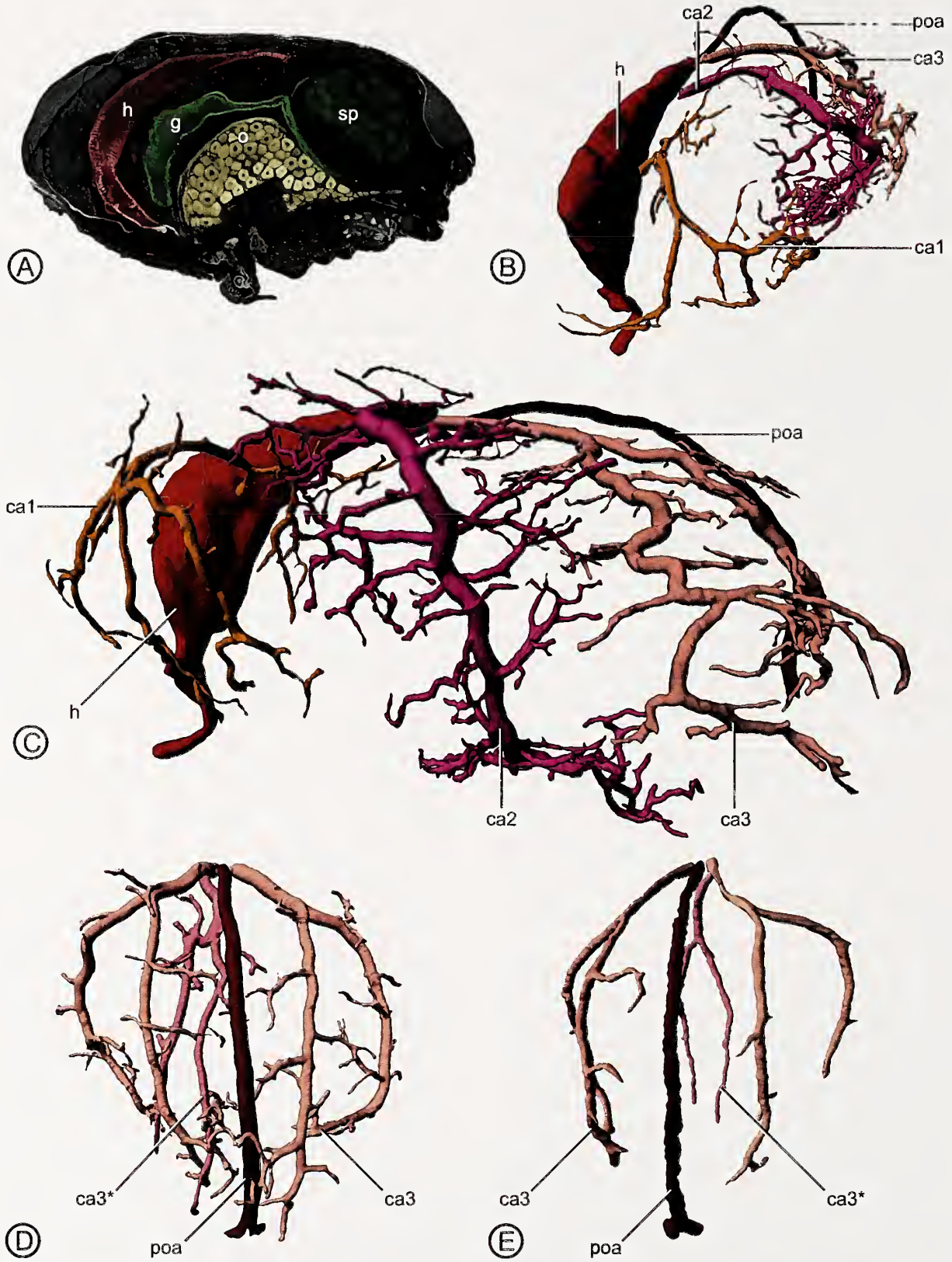

Figure 1.—Hemolymph vascular system in the opisthosoma of *A. diadematus*. PDF version contains interactive 3D content. To activate click on Fig. 1C in Adobe Reader. Rotate object with mouse and see further functionalities in content menu. **A.** Volume rendering of the opisthosoma showing the position of the heart (red), digestive organs (green) and ovaries (beige). Virtual section through the median sagittal plane; lateral view. Specimen-ID: Ad11. **B, C.** Surface rendering showing the hemolymph vascular system in the opisthosoma. Right-sided cardiac artery systems are hidden but are included in the digital 3D content of Fig. 1C. Specimen-ID: Ad01. **B.** Antero-lateral view. **C.** Lateral view. **D, E.** Surface rendering of the posterior aorta system and third cardiac artery systems showing the varying origin of the asymmetric artery (pink);

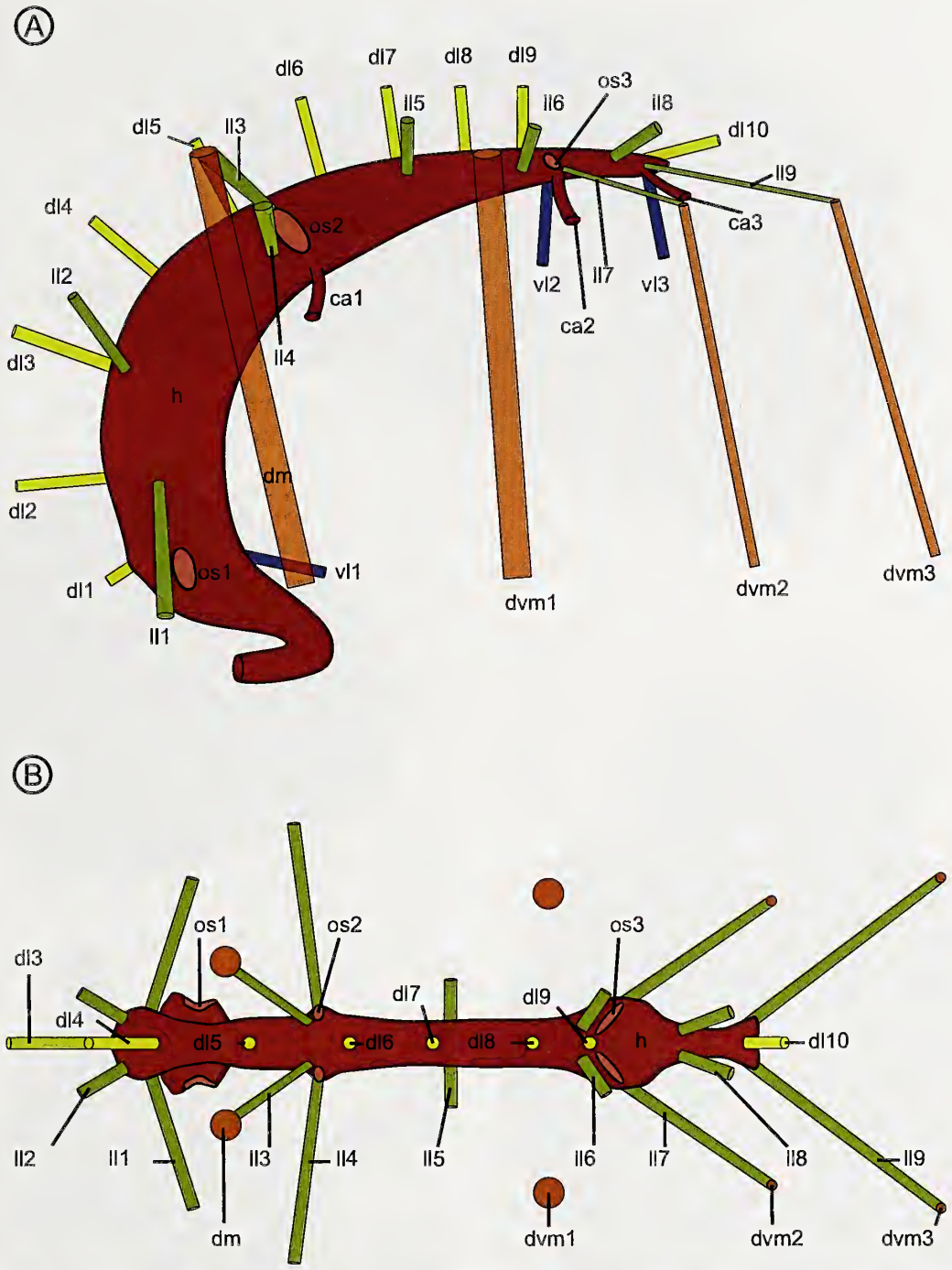

Figure 2.—Schematic drawings of the heart, cardiac artery systems and ligaments in spatial relation to the opisthosomal dorsal and dorso-ventral muscles in *A. diadematus*. **A.** Lateral view. **B.** Dorsal view. ca1–3: first–third cardiac artery; dl1–10: first–tenth dorsal ligament; dm: dorsal muscle; dvm1–3: first–third dorso-ventral muscle; h: heart; ll1–9: first–ninth lateral ligament; os1–3: first–third ostium; vl1–3: first–third ventral ligament.

They supply the anterior region of the prosoma and the chelicerae. Before the cheliceral arteries enter the chelicerae, some major artery systems originate. The optic artery systems (Fig. 3A, B; oa) run dorsally and bend anteriorly towards the eyes. After reaching the median eyes, the optic arteries arch laterally towards the lateral eyes (Fig. 3B, C). The optic artery systems also supply the venom glands (Fig. 3A–C; vg) and the dorsal musculature of the anterior prosoma.

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posterior view. **D.** Asymmetric artery originating from the left third cardiac artery system (ca3*). Specimen-ID: Ad03. **E.** Asymmetric artery originating from the right third cardiac artery system (ca3*). Specimen-ID: Ad45. ca1–3: first–third cardiac artery system; g: gut; h: heart; o: ovary; poa: posterior aorta system; sp: stercoral pocket.

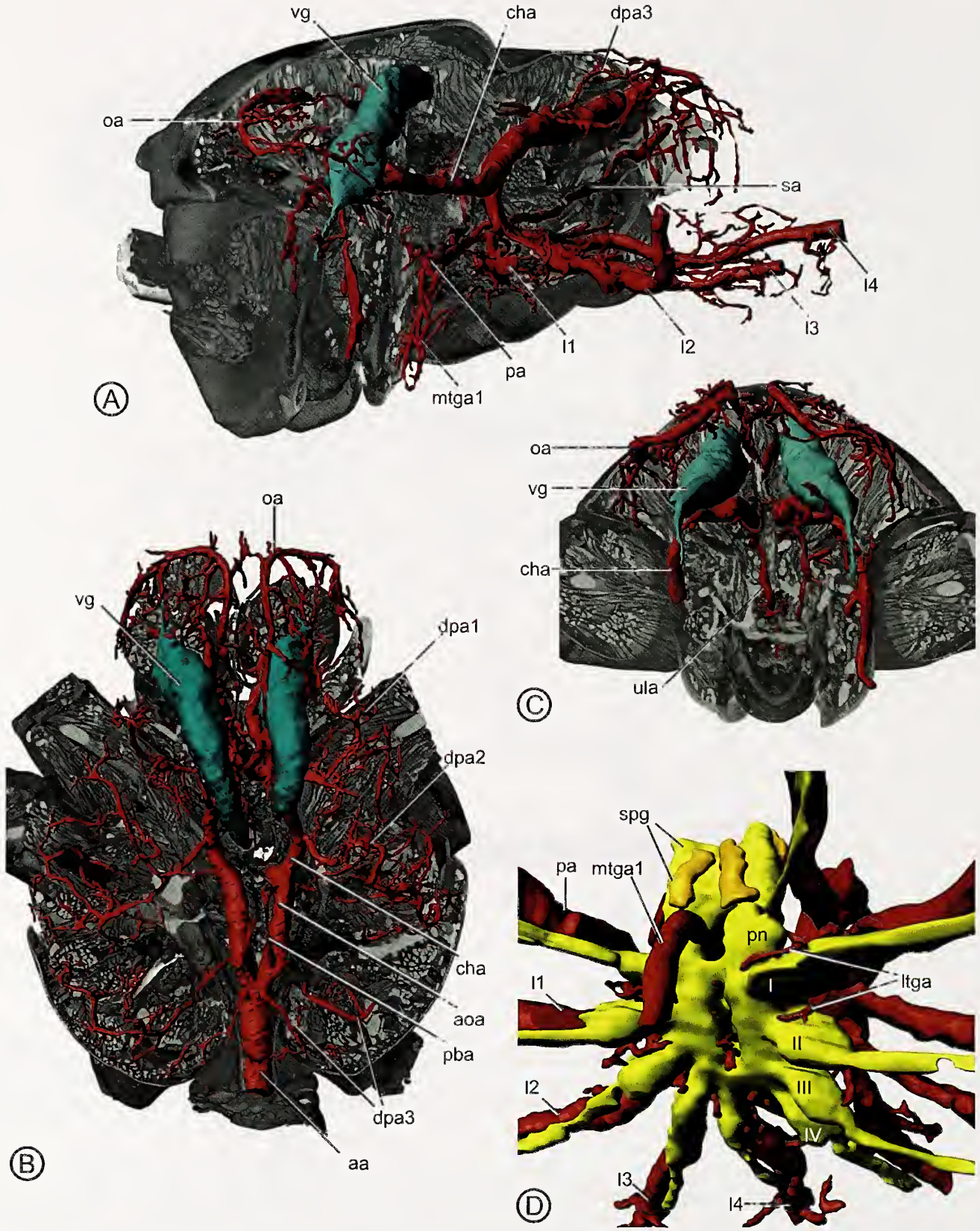

Figure 3.—Hemolymph vascular system in the prosoma of *A. diadematus*. PDF version contains interactive 3D content. To activate click on Fig. 3B in Adobe Reader. Rotate object with mouse and see further functionalities in content menu. **A–C.** Surface rendering of the hemolymph vascular system (red) and the venom glands (cyan) in the prosoma (volume rendering). Specimen-ID: Ad69. **A.** Antero-lateral view; dorsal branching artery systems of the cheliceral artery system are hidden but are included into the digital 3D content of Fig. 3B. **B.** Dorsal view. **C.** Anterior view. **D.** Surface rendering of the hemolymph vascular system and the central nervous system; ventro-lateral view. Specimen-ID: Aq01.

The dorsal musculature of the medial portion of the prosoma is supplied by two paired arteries: the first (dpa1) and the second dorsal prosomal artery systems (dpa2). They originate from the proximal part of the cheliceral arteries (Fig. 3A, B).

The unpaired upper lip artery system (Fig. 3C; ula) originates from one (see 'Variability' below) cheliceral artery. The upper lip artery runs postero-ventrally before it bends ventrally into the upper lip.

Variability: We observed that the origin of the upper lip artery system was not consistent. In five specimens, the upper lip artery originated in the right cheliceral artery and, in another five specimens, it originated in the left cheliceral artery.

Leg artery systems and pedipalpal artery systems: The aortic arches split radially into the four leg artery systems, running dorsally to the subesophageal ganglion (Fig. 3A, D; l1, l2, l3, l4); the first leg artery systems run in an antero-lateral direction, the second leg artery systems in a lateral direction, the third leg artery systems in a postero-lateral direction and the fourth leg artery systems in a posterior direction. There are just a few arteries that branch off in the proximal regions of the leg arteries. Exceptions to this are the pedipalpal artery systems at the first leg arteries, and the lateral transganglionic arteries at each leg artery. The majority of small ramifications at the leg arteries are located inside the leg. These secondary arteries supply the leg musculature. All leg arteries run dorsally along their respective leg nerves (Fig. 3D).

The pedipalpal arteries (Fig. 3A; pa) originate in the proximal region of the first leg arteries and pass the supraesophageal ganglion laterally, nearly at the same level as the esophagus penetrates the supraesophageal ganglion (Fig. 3D). The pedipalpal arteries are located dorsally on the pedipalpal neuropils. The pedipalpal arteries divide and these arteries supply the large coxal podomere and the distal pedipalpus, respectively (Fig. 3A; interactive 3D content).

Supraneural artery system: The unpaired supraneural artery system originates at the medial side of one aortic arch, or in the transient area to the most proximal region of one fourth-leg artery. The supraneural artery runs posteriorly along the median line of the body resting on the dorsal side of the opisthosomal nerve. The supraneural artery represents the origin of the posterior group of the median transganglionic arteries (mtga) (further details on mtga are provided below in "Supply of the central nervous system"). This artery traverses the pedicel ventrally to the opisthosomal nerve, however, it seem that it does not enter the main part of the opisthosoma, as the injected resin stopped abruptly at the posterior end of the pedicel.

Variability: The origin of the supraneural artery system can either be found on the left (in six specimens) or the right (in three specimens of *A. diadematus* and in the specimen of *A. quadratus*) aortic arch. No correlation was found between the originating sides of the first median transganglionic artery and the supraneural artery; all four possible combinations were present in *Araneus* (Fig. 4).

Supply of the central nervous system (prosomal ganglion): The supraesophageal ganglion is supplied by two pairs of ventral branching arteries originating at the ventro-medial side of the cheliceral arteries (Fig. 5; vb1, vb2). They run postero-ventrally into the supraesophageal ganglion and end there.

The subesophageal ganglion is supplied by two groups of arteries: the median transganglionic arteries (mtga) and the lateral transganglionic arteries (ltga).

The unpaired median transganglionic arteries penetrate the subesophageal ganglion along the midline in a regular pattern. They run to the ventral side of the subesophageal ganglion where they end openly (Fig. 3D; mtga). The number of observed, i.e., injected median transganglionic arteries varied from 5 to 12 (in 4 specimens of *A. diadematus* and the specimen of *A. quadratus*; for a more detailed analysis, see 'Discussion'). Despite the variety in the number of median transganglionic arteries, our study detected differing sites of origin: the arteries branch off the first median transganglionic artery, off one aortic arch or off the supraneural artery.

The first median transganglionic artery (Fig. 3A; interactive 3D content; mtga1) originates from the medial side of one aortic arch dorsally to the subesophageal ganglion. This artery penetrates the prosomal ganglion and runs ventrally along the esophagus (Fig. 3D). Posterior to the pharynx, the first median transganglionic artery bends ventrally and enters the lower lip with numerous ramifications. A varying number of median transganglionic arteries originate from the first median transganglionic artery (for further details see 'Variability' below).

Variability: The origin of the first median transganglionic artery system is either on the left (in four specimens of *A. diadematus* and in the specimen of *A. quadratus*) or the right aortic arch (in seven specimens).

A more detailed account of the median transganglionic arteries of two specimens is now presented, because a more generalized description was not possible:

Specimen Ad86 (with twelve detected mtga): The first median transganglionic artery originates from the right aortic arch. The second to seventh anterior median transganglionic arteries originate from the larger first median transganglionic artery. The subsequent five posterior median transganglionic arteries originate from the supraneural artery. The supraneural artery originates from the left aortic arch (Fig. 5A).

Specimen Aq01 (with eleven detected mtga): The first median transganglionic artery originates from the left aortic arch. The second to third anterior median transganglionic arteries originate from the larger first median transganglionic artery. The subsequent two median transganglionic arteries originate from the right aortic arch. The subsequent six median transganglionic arteries originate from the supraneural artery (Fig. 5B). The supraneural artery originates from the right aortie arch.

The six pairs of lateral transganglionic arteries (ltga) arise at the leg arteries and pedipalpal arteries. They run ventrally

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I–IV: first–fourth walking leg neuropil; aa: anterior aorta system; aoa: aortic arch; cha: cheliceral artery system; dpa1–3: first–third dorsal branching artery system; pba: peribuccal artery system; l1–4: first–fourth leg artery system; ltga: lateral transganglionic arteries; mtga1: first median transganglionic artery system; oa: optical artery system; pa: pedipalpal artery system; vg: venom glands; pn: pedipalpal neuropil; sa: supraneural artery system; spg: supraesophageal ganglion; ula: upper lip artery system.

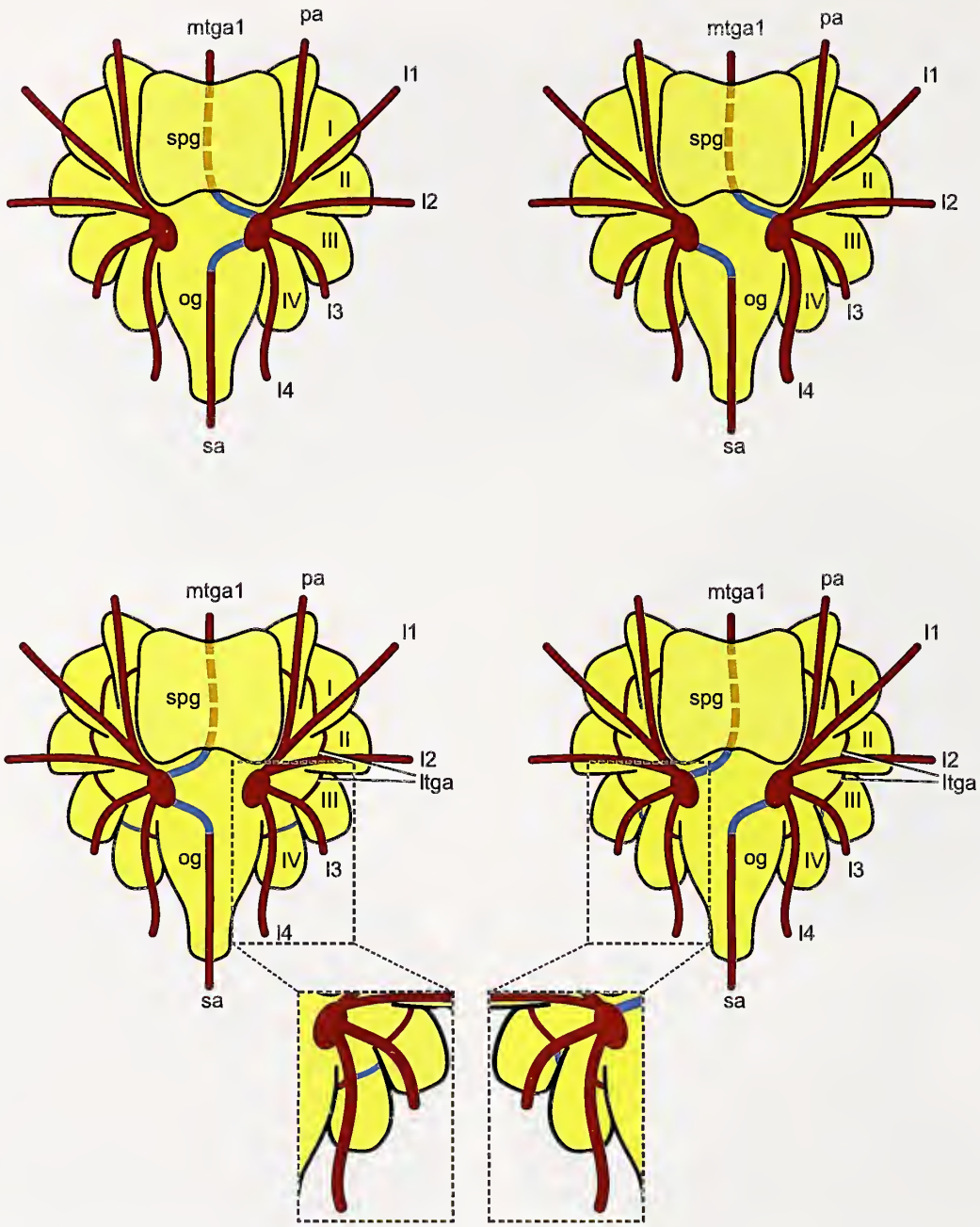

Figure 4.—Schematic drawings illustrating intraspecific variability in the origin (blue) of artery systems (red) in the region of the central nervous system (yellow); dorsal view. The four schemes show all possible combinations of origins of the first median transganglionic artery and the supraneural artery system. The lower row of schemes also shows the different origins of the lateral transganglionic arteries. I–IV: first–fourth walking leg neuropil; l1–4: first–fourth walking leg artery; ltga: lateral transganglionic arteries; mtga1: first median transganglionic artery; og: opisthosomal ganglion; pa: pedipalpal artery; sa: supraneural artery; spg: supraesophageal ganglion.

between the lateral processes of the subesophageal ganglion (Fig. 3D; ltga). At the ventral side of the subesophageal ganglion, all lateral transganglionic arteries bend medially and end openly. The lateral transganglionic arteries arising from the pedipalpal and first to third leg arteries always have their origin at the anterior side. Thus the first lateral transganglionic arteries (originating from the pedipalpal arteries) lie anterior to the pedipalpal neuropil. The subsequent lateral transganglionic arteries originating from the first to third walking leg arteries conform to the same pattern. The last two pairs of lateral transganglionic arteries are an exception to this pattern, surrounding the fourth leg neuropil anteriorly and posteriorly. So the most posterior lateral transganglionic arteries, between the fourth leg neuropil and opisthosomal ganglion always originate at the posterior side of the fourth leg artery (Fig. 4).

Variability: The lateral transganglionic arteries running between the third and fourth leg neuropils can either originate at the posterior side of the third leg artery (in one specimen) or at the anterior side of the fourth leg artery (in six specimens and one specimen of *A. quadratus*), but always symmetrically in each specimen (Fig. 4).

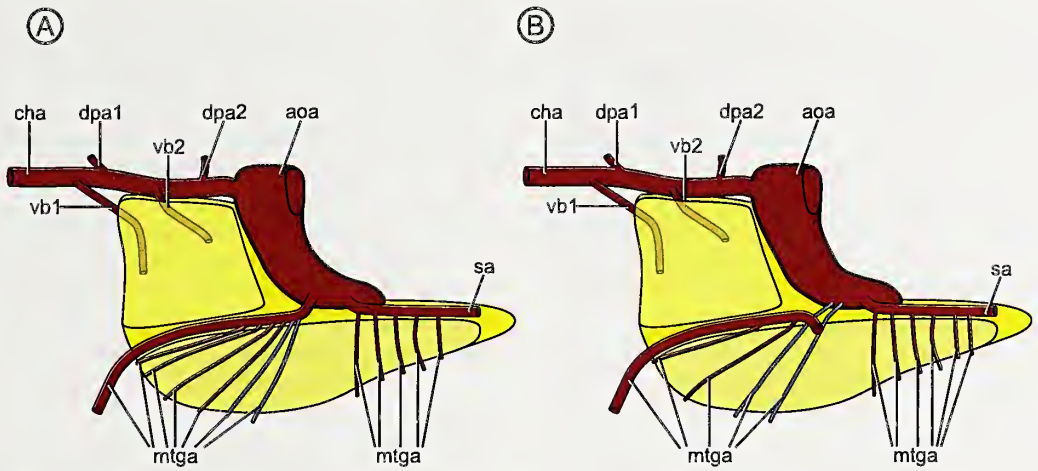

Figure 5.—Schematic drawings illustrating intraspecific variability (blue) concerning the median transganglionic arteries in the hemolymph vascular system (red) in the region of the central nervous system (yellow); lateral view. Exemplified on the specimens **A.** Ad86 and **B.** Aq01. aoa: right aortic arch (partially); cha: cheliceral artery system (partially); dpa1–2: first–second dorsal branching artery; mtga: median transganglionic arteries; sa: supraneural artery system; vb1–2: first–second ventral branching artery.

There are further arteries supplying the region between the subesophageal ganglion and the sternum, which are not in direct contact with the central nervous mass. These arteries stem from all four leg arteries before they enter the legs, and from the first median transganglionic artery before it enters the lower lip (not shown).

DISCUSSION

Comparative morphology of the HVS in the prosoma.—Only a handful of studies describing arteries in *Araneus diadematus* currently exist, and there is indeed only one study (Schneider 1892) that describes prosomal artery systems. Generally within Araneae, the course of the anterior aorta and its two aortic arches is corresponding (Schneider 1892; Causard 1896; Gerhardt & Kaestner 1938; Crome 1952; Huckstorf et al. 2013, 2015). However, in the following section, differences in the branching patterns of prosomal vascular systems in Araneae are discussed.

Our study indieates that the dorsal prosoma in *A. diadematus* is supplied by five paired artery systems: the optic artery system (oa), three dorsal prosomal artery systems (dpa1–3) and the peribuccal artery system (pba). This indicates two more dorsal artery systems than previously described by Schneider (1892). As Schneider (1892) correctly identified, the anteriormost off-branching artery system (her "nv", fig. 28) supplies the venom glands. However, we found differences with respect to the orientation of this artery system and the region it supplies. A large artery of both artery systems bends anteriorly by flanking main parts of the venom gland (see Fig. 3B), and not posteriorly as described by Schneider (1892). Indeed, only a small part of the venom glands is supplied by a separate, posteriorly-running artery. Furthermore, this artery system supplies the whole anterior region on each side of the prosoma from the median eyes to the lateral eyes and is therefore termed the optic artery system here. No such description of this course of the optic artery system in *A. diadematus* existed until now. However, similar optic artery systems have been described for other species (*Tegenaria* sp.: Schneider 1892; *Agelena labyrinthica* (Clerck, 1757): Causard 1896).

Apart from the optic artery system, two further artery systems are deseribed by Schneider (1892); a dorsal prosomal artery system posterior to the optic artery system (which cannot definitely be equated to the first or the second dorsal prosomal artery system described in the present study), and an artery system supplying the dorsal dilatator muscles of the stomach, which can be matched to the herein deseribed peribuccal artery system. Our study therefore identifies two additional dorsal prosomal artery systems in *A. diadematus*, and describes them here for the first time; one originates from the cheliceral artery (Fig. 3B; 3D content; dpa1 or dpa2), and the other – the third dorsal prosomal artery system (Fig. 3A, B, 3D content; dpa3 – originates from the anterior aorta after its first division. A comparable number of dorsal prosomal arteries with comparable supplied regions were described in *Cupiennius salei* (Keyserling, 1877) (Huckstorf et al. 2013). In *C. salei*, two paired dorsal prosomal arteries (their "dorsal branches of the cheliceral artery") originate from the cheliceral artery, as in *A. diadematus*. The third dorsal prosomal artery system in *C. salei* (their "dorsal artery of the anterior aorta") exhibits notable differences as it is unpaired, and originates posteriorly to the first ramification of the anterior aorta.

Furthermore, we can demonstrate that the origin of the upper lip artery system lies anterior to that of the optic artery system on the cheliceral artery (6 specimens). These results throw new light on Schneider's (1892) study, which previously described the origin of the upper lip artery system to be posterior to the optic artery system.

Arterial supply of the prosomal ganglion: Two pairs of arteries, which have not been identified before in *A. diadematus*, supply the supraesophageal ganglion. In *C. salei*, however, three pairs of arteries originating from the cheliceral artery have been shown to supply this region of the brain (Huckstorf et al. 2013). It remains unclear however, whether the number of supraesophageal arteries contains phylogenetic information, or whether it correlates with size of the supra-esophageal ganglion.

With regards to the subesophageal ganglion, the lateral transganglionic arteries have been described for *A. labyrinthica* by Causard (1896), *C. salei* by Huckstorf et al. (2013) and for *Kukulcania hibernalis* (Hentz, 1842), *Austrochilus forsteri* Grismado, Lopardo & Platnick, 2003 and *Hickmania troglodytes* (Higgins & Petterd, 1883) by Huckstorf et al. (2015). The origin of the lateral transganglionic arteries in these species exhibit the same pattern: namely one lateral transganglionic artery that originates anteriorly in the proximal region of the pedipalpal artery, one lateral transganglionic artery that originates anteriorly in the first to fourth walking leg artery, respeetively and – at the fourth walking leg artery – one lateral transganglionic artery that originates posteriorly. All lateral transganglionic arteries run between adjacent lateral neuropils. This pattern was not consistent in *A. diadematus* since the origin of the fifth lateral transganglionic arteries is subjeet to intraspecific variability (Fig. 4). The alternating pattern where the fifth lateral transganglionic arteries originates posteriorly from the third walking leg artery has not been described in Araneae.

The median transganglionic arteries in Araneae generally originate from five arteries connecting the right and the left aortic arches or the supraneural artery (Schneider 1892; Causard 1896; Huckstorf et al. 2013). This pattern has also been described in scorpions (Klußmann-Frieke et al. 2012, 2014), Amblypygi and Uropygi (Klußmann-Fricke & Wirkner in press). In *Araneus,* connecting arteries are missing so the origin of the median transganglionic arteries can be found at one aortic arch, the first median transganglionic artery or the supraneural artery.

Another variation found in median transganglionic arteries in different specimens (see results) points to a different rather teehnical problem. The injeetion method itself may lead to different results concerning the median transganglionic arteries due to their relative small diameters, i.e., in some specimens some of the median transganglionic arteries may have not been filled with resin. We therefore generally refrained from taking numerical variation into account in this study. All variation accounted here relates to structural differences. We therefore postulate that 12 median transganglionic arteries (the maximal number counted) occur in *A. diadematus*, but this figure is still lower than the results presented by Schneider (1892), who counted 13 median transganglionic arteries (including her "labiale post." (lp) supplying the lower lip, see below). Taking other investigations into aecount, it appears that the number of median transganglionic arteries varies between spider species from, for example, *Tegenaria* sp. (Schneider 1892) and *A. labyrinthica* (Causard 1896) with 13 to 12 median transganglionic arteries in *C. salei* (Huckstorf et al. 2013). The lowest number hitherto presented is for *Argyroneta aquatica* (Clerck, 1757) with six median transganglionic arteries (Crome 1952). Whether or not quantitative intraspecific variability also occurs in median transganglionic arteries still needs to be tested in a future study.

The arterial supply of the lower lip in *A. diadematus* (Fig. 3A) and in *A. quadratus* is peculiar, since the corresponding artery originates from one aortic arch and not from the chelieeral artery, as in other spiders (Causard 1896; Crome 1952; Huckstorf et al. 2013). This artery represents the elongated first median transganglionic artery (Schneider 1892). This can be deduced from its origin and its eourse through the subesophageal ganglion on the ventral side of the esophagus. We can therefore conclude that the lower lip artery (originating from the cheliceral artery) has been redueed in *Araneus*, while the hemolymph supply is taken over by the first median transganglionic artery.

The first median transganglionic artery and the supraneural artery can originate from the right or the left aortic arch, respectively. As illustrated in the schematic drawings (Fig. 4), all four possible combinations were present in *Araneus* (see Fig. 4). No eorrelation was found between the originating sides of the first median transganglionic artery and the supraneural artery.

Comparative morphology of the HVS in the opisthosoma.— *Heart:* The posterior aorta of most spiders is unbranched except for a terminal bifurcation close to the spinnerets (Fig. 1D, E) (Causard 1896; Huckstorf et al. 2013, 2015), and *A. diadematus* is no exception to this. Indeed, the only recorded account of any variation is in *Dysdera*, which displays a more anteriorly branched posterior aorta, a shortened heart and a lower number of eardiac artery systems (Schneider 1892; Causard 1896).

Ostia: Spider taxa show notable differences when comparing both the number and arrangement of ostia, and the origin of cardiac arteries. With regards to the ostia, studies by Petrunkevitch (1933) have shown how the number of pairs of ostia can vary from five (in Liphistiidae) to two. In the Mygalomorphae and Hypoehilidae, the heart is equipped with four pairs of ostia. Within the Mygalomorphae, the number of ostia is reduced to three pairs in the taxa Barychelidae and Migidae. There are more independent reductions to three pairs of ostia in Filistatidae, Synspermiata and Entelegynae (see Huckstorf et al. 2015). Several taxa independently exhibit even fewer ostia – down to two pairs in taxa such as Cybaeidae, Dysderidae, Segestriidae, etc. (see Petrunkevitch 1933 for a comprehensive list). The number and position of the three ostia found in *A. diadematus* fits the ground pattern of Entelegynae and reinforces available data (Causard 1896; Willem 1917).

Cardiac artery systems: Available literature is less expansive on the subject of the cardiac arteries. However, we can find evidence of a reduction from five pairs in Liphistiidae (Millot 1933) down to two pairs in *A. aquatica* (Crome 1952) and *Segestria* sp. (Schneider 1892; Causard 1896). In Hypochilidae and Austrochilioidea the number of ostia is reduced to four, of which the posterior three are assoeiated with a eardiac artery system (Huckstorf et al. 2015). The heart of *A. diadematus* possesses three paired ostia and three paired cardiac arteries (Fig. 2). The origin of the first and second cardiac arteries is located ventrally to the subsequent two ostia. The third cardiac artery originates in the posterior region of the heart and is not associated with a pair of ostia. This arrangement of ostia and cardiac arteries corresponds to that exhibited in Filistatidae, Synspermiata and Entelegynae (Causard 1896; Willem 1917; Huckstorf et al. 2013, 2015). The results of this study regarding the position of ostia and origin of cardiac arteries confirm the results of Causard (1896) and Willem (1917) for *A. diadematus* and contradict former descriptions of six pairs of cardiac arteries (Blanchard 1849). In comparison

the evolutionary reduction in number of ostia and of cardiae arteries show different patterns; for a detailed discussion see Huckstorf et al. (2013).

The most complex aspect of the opisthosomal HVS in spiders, the branching pattern of the cardiac artery systems, has so far been illustrated in just a few cases (Schneider 1892; Causard 1896; Crome 1952; Huckstorf et al. 2013, 2015). The only results previously available for cardiac arteries in *A. diadematus* (Blanchard 1849) have to be dismissed: the number of cardiac arteries stated makes it unreliable. The present study therefore constitutes a novel and up-to-date insight into the branching pattern of the three cardiac artery systems in *A. diadematus*.

The main artery and most of the smaller arteries of all three cardiac artery systems in *A. diadematus* run in close proximity to the cuticle to the ventral side of the body. There is only a small amount of overlap in the body regions (anterior-posterior axis) supplied by each cardiac artery system. This picture differs in *Agelena labyrinthica* where the second cardiac artery system mainly remains in the interior opisthosoma (Causard 1896). The periphery of the mid-opisthosoma is supplied by arteries extending anteriorly from the third cardiac artery system (Causard 1896). Differences can also be found in *C. salei* when comparing the regions supplied by the cardiac artery systems. In this species, the second cardiac artery system supplies the bulk of the ventral opisthosoma. Conversely, the ventral extension of the first and third cardiac artery system in the anterior and posterior part of the opisthosoma is redueed, respectively (Huckstorf et al. 2013). To date, only a few studies are available that illustrate the cardiac artery systems in detail. Therefore, the identification of morphological or phylogenetic patterns is hampered and has to be left open at this point.

Ligaments: Forming a coherent account of the ligament pattern attached to the heart within the opisthosoma in *A. diadematus* is not easy. Existing literature details differing numbers for each of the three different types of ligaments (Schimkewitsch 1884; Schneider 1892; Causard 1896; Willem 1917). The most extensive study so far on ligaments by Causard (1896) describes 11 dorsal ligaments, eight lateral ligaments and five ventral ligaments. Considering the dorsal and lateral ligaments this description differs from our findings of ten dorsal ligaments and nine lateral ligaments. However, it is obvious that there is an equal number of ligaments overall in both studies (19 dorsal and lateral ligaments). By comparing both sequences, it appears likely what we describe as the eighth lateral ligaments, was interpreted as the tenth dorsal ligaments by Causard (for further descriptions of the set of ligaments in other species see Causard 1896; Millot 1933; Crome 1952; Huckstorf et al. 2013). Because the results are too varied and are taken from an insufficient number of species, we are unable to undertake an expedient discussion here. However, further investigations on this topic are obviously needed as the ligament patterns seem to reveal information with regards to the shortening of the heart (Huckstorf et al. 2013, 2015).

Intraspecific variability.—One of the aims of our study was to highlight intraspecific variability within the HVS of *A. diadematus* by studying 33 specimens. This number far exceeds common morphological practice, in which just a few specimens are analyzed. Comparing our study with other investigations into the HVS in arthropods (Keiler et al. 2013, 2015 a, b; Huckstorf et al. 2015; Göpel & Wirkner 2015), we can conclude that there are four major patterns of intraspecific variability:

i. Variability of the course of arteries
ii. Variability of the number of off-branching arteries
iii. Variability of arterial origins
iv. Variability of the occurrence of anastomoses

Apart from the last phenomenon, all of the above-listed patterns of variability occur in the HVS of *A. diadematus*. However, in comparison to other spiders, we found that the HVS in *Araneus* differs remarkably by the tendency to reduce paired origins of arteries arranged along the body midline to an unpaired, unilateral origin. These unilateral origins are regions in which we frequently observed intraspecific variability.

Although these forms of intraspecific variability occur, there are many features and patterns that are consistent across the specimen range. These stable patterns can reliably be used for character conceptualization for phylogenetic and evolutionary analyses (Richter & Wirkner 2014).

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Limb loss and limb regeneration of crab spiders *Misumena vatia*

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Abstract. Limb loss presents an interesting paradox: although it may permit escape from a potentially lethal situation, it may result in subsequent fitness-lowering consequences. Some studies have found costs of limb loss; others have not. If costs are high, they may dictate against retaining the ability to drop an appendage. I use a large data set derived from a long-term study of the crab spider *Misumena vatia* (Clerck, 1757) to investigate the role of several size- and time-related factors in evaluating the cost of losing variable numbers of legs, as well as of growing replacements. Specifically, do limb loss and regeneration affect condition, and do the results differ with sex and age? I focused on adult males because of their high frequency of forelimb loss, including loss of multiple limbs. Numbers of missing adult male forelimbs were correlated with date captured and mass (corrected for number of missing forelimbs), suggesting that the spiders lost forelimbs continually over the summer and that they reached progressively poorer condition than intact individuals, judged by a disproportionate loss in body mass. The frequency of forelimb loss by penultimate males matched that of adult males, but females and juveniles lost less than $1/10^{th}$ as many forelimbs as males. Males possessed many fewer partially regenerated forelimbs than missing forelimbs, but these frequencies significantly exceeded those for females and younger juveniles. Some information suggested that additional costs arose from the regeneration of forelimbs.

Keywords: Male-female difference, male-juvenile difference, predation, sexual dimorphism, Thomisidae

Considerable controversy exists over the consequences of limb loss among many-limbed invertebrates such as spiders and crustaceans. Although some studies have found little or no measurable effect of limb loss on survival or fitness (Guffy 1998; Johnson & Jakob 1999); others have demonstrated such a link, which may have major effects, including both prey capture and escape from predators (Maginnis 2006a; Steffenson et al. 2014). Most limb losses in these forms result from autotomy, a voluntary, nervously mediated defensive response induced by external stimuli (Wilkie 2001; Fleming et al. 2007). A widespread phenomenon, members of at least five invertebrate phyla, 14 classes and 36 orders readily shed limbs (Fleming et al. 2007), and many have retained the ability to regenerate lost parts under certain conditions (Bely & Nyberg 2009; Foelix 2011). Shedding a limb provides a convenient way to escape predation or other contingencies, but the act may have negative future consequences for an individual (Brueseke et al. 2001; Lutzy & Morse 2008). In response to loss, some species regenerate limbs, but this ability is not universal (Randall 1981; Maginnis 2006a), and the utility of this trait is open to question in some species (Maginnis 2006b; Lutzy & Morse 2008). In particular, many of these studies provide little detail that might provide broader insight into the overall advantages of the ability to shed a limb. Although shedding a limb, as opposed to shedding a life, is clearly advantageous in the short term, what success do the survivors enjoy? Do they obtain any fitness benefits that would separate them from those preyed upon, and if so, how mueh? The data set assembled here allows in-depth evaluation of this question. For instance, do losses of one, two or three limbs change the probability of future success?

Here I present data on limb loss and subsequent regeneration from a large sample of crab spiders, *Misumena vatia* (Clerck, 1757) (Thomisidae) gathered over 13 seasons, which provide insight into the possible relationship of several potentially key variables to limb loss. More specifically, do size (carapace width), mass, mass correeted for limb loss, date of capture, date of death, year, or collection site vary with limb loss? And, what relationship does regeneration have to these variables, any of which might provide insight into the fitness consequences of shedding limbs and attempts to regenerate them? I chose these variables because they allow key insight into the possible effects of size on limb loss and how limb loss affects body condition. The high frequency also provides the opportunity to gather adequate numbers of individuals for analysis. Do these relationships change or do they remain predictable and constant? And, is it worth trying to regenerate a limb?

Male *Misumena* Latreille, 1804 and related species lose limbs with a high frequency (Dodson & Schwaab 2001; Lutzy & Morse 2008). High mobility is an important trait, and the loss of forelimbs seriously compromises the spiders' locomotor capacity (Lutzy & Morse 2008). Although foeusing on adult males, I compare them where possible with penultimate males, adult and penultimate females, and younger juveniles. One of the most highly sexually dimorphic of terrestrial animals (LeGrand & Morse 2000; Morse 2007), *Misumena* provides an excellent opportunity to make both intrasexual and intersexual comparisons. Forces acting on individuals of such strikingly different sizes may constrain the opportunity for further change within one sex or the other. To the best of my knowledge, no similar comparisons in extremely sexually dimorphic species have been made (Maginnis 2006a; Fleming et al. 2007). This work also expands on the results of an earlier three-year study (Lutzy & Morse 2008) of adult males. I then comment on the significance of these results for interpreting the present pattern of forelimb loss and regeneration in *Misumena*.

METHODS

Study area.—I collected *Misumena* in an old field at the Darling Marine Center in South Bristol, Lincoln Co., Maine (43°57′N, 69°33′W), and at several other locations along

roadsides within 5 km of the Darling Center. These locations are not managed except for being mown yearly. The sample from the Darling field is a probable composite of the other collection sites, because egg masses of females taken from the other sites include those used to rear spiderlings for a wide range of field experiments conducted over the period encompassed by this study (2000–2012). Numbers of individuals from three other collection sites permitted individual treatment as separate samples; those from 14 other locations were pooled into a last sample. These five samples were numbered 1–5.

The sites contain large numbers of flowers upon which *Misumena* hunt as sit-and-wait predators. I inspected flowers and collected spiders as I found them. Principal flower species included common milkweed *Asclepias syriaca*, oxeye daisy *Leucanthemum vulgare*, common buttercup *Ranunculus acris*, black-eyed susan *Rudbeckia hirta*, eommon St. Johnswort *Hypericum perforatum*, early goldenrod *Solidago juncea* and Canada goldenrod *S. canadensis*. Although this study focuses on adult males, I simultaneously captured penultimate males, penultimate females, adult females and earlier instars. I used many of these spiders in a wide variety of experiments not central to the present study. The data set includes individuals from 2000 and 2001 in Lutzy & Morse (2008), but does not include results from 1999 because those data lack several variables treated here.

The species.—*Misumena vatia* is a highly sexually dimorphic sit-and-wait predator that frequents hunting sites in flowers (described in detail in Morse 2007). Males are small and extremely mobile, weighing from 2.5 to 8.0 mg with two pairs of long anterior limbs (forelimbs) over twice the length of the hind limbs. Males do not gain significant amounts of mass as adults; hence, initial weighing of these individuals provides an adequate measure of adult mass. The highly mobile adult males search for unmated females and often guard large penultimate females that will soon molt into adults, at which point they will mate (Hainsworth & Morse 2000).

Penultimate males generally resemble adult males, though with somewhat shorter forelimbs and larger abdomens. Both penultimate and adult females are mueh larger and more sedentary than the adult males. Large penultimate females weigh 25 mg or more, and gravid adult females weigh as much as 400 mg or more. Most juveniles (early instars) sampled weighed 5–10 mg, near the size range of the males.

Procedure.—I captured the spiders during visits to flowers at the various collection sites. I inspected flowers for the presence of crab spiders, captured these individuals and placed them in 7-dram vials (5 cm tall, 3 cm diameter). I used the spiders in a wide variety of experiments, which I have reported elsewhere (e.g., Morse 2007, 2014). I retained approximately half of the adult males in 7-dram vials through senescence, feeding them every 2–3 days with small moths, flies and mosquitoes. I subsequently used the remainder in field experiments in other projects, and often lost these individuals in the process. I gathered all the data presented, other than date of death, at the time of capture; thus, they are not subject to potential changes brought about by retention in the laboratory.

I first categorized males in terms of their number of missing forelimbs, taking care not to include any individuals that had lost limbs during capture. Then I measured the carapace width of each individual to obtain a measure of overall size independent of mass. I also weighed each individual with a Denver Microbalance (Denver Instrument Company Model A-200DS: Arvada, Colorado, USA), then calculated an estimated mass for individuals missing forelimbs, using an earlier measure of the mass of forelimbs, 8.2% of the body mass (Lutzy & Morse 2008). Thus, I added 8.2% to the measured mass of individuals missing one forelimb, 16.4% to those missing two forelimbs, and 24.6% to those missing three forelimbs. Because the mass of the first and second pairs of limbs was similar in an earlier study (Lutzy & Morse 2008), I added the same mass for each missing forelimb. In all, I obtained a sample of 609 adult males with normal-sized forelimbs, including those with one or more missing forelimbs. Seventy (11.5%) of these individuals lacked one forelimb, 32 (5.3%) lacked two forelimbs, and 8 (1.3%) lacked three forelimbs. I also obtained information about capture site, year, date of capture and date of death. I compared forelimb loss of the adult males with that of penultimate males, penultimate females, and adult females captured at the same times as the adult males and used in a wide range of unrelated experiments.

Additionally, 24 adult males had one or more small regenerating forelimbs no more than half the length of normal-sized forelimbs. I eollected the same data for these individuals as for those lacking forelimbs. I also calculated expected intact masses for the individuals with short forelimbs, assuming that the mass of these limbs weighed one-fourth of their "normal" weight. Only two individuals lost one of their small posterior limbs, and I did not separate these individuals from the others.

To evaluate the costs of forelimb loss and regeneration I used the above-noted data on date of capture, date of death, year, and capture site. These variables all provide insight into the consequences of forelimb loss. Date of capture provides an opportunity to determine whether losses continue as the season progresses. Date of death provides the opportunity to compare individuals from the field with those spared predation, competition and other contingencies in the laboratory. A decline in numbers infers mortality in the field. Significant differences in year or capture site would complicate the process of pooling data sets.

Analysis.—I analyzed the relation of limb loss and presence of short limbs to carapace width and mass with one-way ANOVAs. I also tested capture site, year, date of capture and date of death (in the laboratory) in relation to forelimb loss with ANOVAs. I used analysis of covariance (ANCOVA) to evaluate further the effects of capture site, year, date of capture and date of death on the size of males missing varying numbers of forelimbs. I compared frequencies of forelimb loss and short forelimbs among the different age and sex combinations using G tests and the relationship of carapace width and mass with linear regression. Because I lacked data for every variable measured or tested on each individual, n's differ in some of the analyses. Analyses were carried out in R Version 2.13.0 (R Development Core Team 2011).

RESULTS

Missing forelimbs.—A substantial proportion of the adult male spiders lost one or more forelimbs (110 of 609: 18.1%), a

Table 1.—Characteristics of adult male *Misumena vatia* missing 0–3 forelimbs (mean ± SD), and results of ANOVAs. [1] Predicted mass of individual assuming it still possessed all forelimbs; [2] Julian days (30 June = 180); [3] Halfway between 2004 and 2005 = 4.5, etc.; [4] Collection sites numbered 1–5 (see Methods), with non-significance denoting no difference among sites.

Variable					*F*	*df*	*P*
Number of missing forelimbs (*n*)	0 (491)	1 (70)	2 (32)	3 (8)			
Carapace width (mm)	1.47±0.149	1.46±0.151	1.43±0.132	1.47±0.144	2.09	1,599	0.15
Mass (mg)	4.58±1.540	4.20±1.415	3.44±1.010	3.06±1.066	26.45	1,599	<0.0001
Mass corrected[1]	4.58±1.540	4.54±1.533	4.01±1.182	3.76±1.357	5.1	1,599	0.024
Date of capture[2]	172.1±10.66	173.2±13.24	173.8±10.59	181.6±15.05	4.53	1,599	0.034
Date of death[2]	203.4±18.11	201.5±21.96	201.6±16.48	207.6±25.42	0.11	1,300	0.74
Year[3]	4.5±4.20	4.4±4.25	5.6±4.65	5.4±4.57	1.4	1,599	0.24
Collection site[4]	2.7±1.57	2.9±1.56	2.9±1.68	3.3±1.91	1.56	1,599	0.21

result that did not significantly exceed the proportion of penultimate males missing a forelimb (6 of 52: 11.5%; $G_1 = 1.84$, $P > 0.1$). The adult male proportion greatly exceeded those of both penultimate (7 of 336: 2.1%; $G_1 = 64.35$, $P < 0.0001$)) and adult females (14 of 862: 1.6%; $G_1 = 97.13$, $P < 0.0001$). The frequency of forelimb loss in the penultimate males also exceeded that of both penultimate and adult females ($G_1 = 8.27$, $P < 0.01$; adults: $G_1 = 11.72$, $P < 0.001$). Numbers of missing forelimbs of unsexed earlier instars closely resembled those of females (4 of 227: 1.8%), significantly less than both adult ($G_1 = 88.89$, $P < 0.0001$) and penultimate ($G_1 = 9.60$, $P < 0.01$) males.

Body size (carapace width) did not differ significantly with the loss of one or more forelimbs, with the four forelimb-loss classes (0 – 3) exhibiting similar carapace widths at all of the sites (Table 1). As predicted, individuals missing progressively more forelimbs weighed less than those with fewer missing forelimbs (Table 1). However, after correcting masses for forelimbs lost, those with missing forelimbs were still significantly lighter than intact ones (Table 1). Although differences in mass between intact individuals and those with a single missing limb (correeted for estimated mass of that missing forelimb) were modest, suggesting a relatively minor effect, those between one and two missing limbs showed a several-fold decrease in mass, which was further extended in those missing three limbs (Table 1). Carapace width and mass (corrected) were nevertheless highly correlated (linear regression: $R^2 = 0.643$, $F_{1,599} = 1083$, $P < 0.0001$), accounting for roughly two-thirds of the total variance.

Among other variables measured, proportions of individuals missing forelimbs increased as the season progressed (date captured) (Table 1), although the majority of forelimb loss had already occurred by the first measure, early in the season. Neither date of death, carapace width, year, nor site of collection differed signifieantly in relation to forelimb loss (Table 1), allowing me to pool these data sets. Use of date of capture, date of death, year and collection site as covariates resulted in only one change in the relationship between size and forelimb loss: site had a moderately significant effect ($F_{3,298} = 2.81$, $P = 0.040$).

Short forelimbs.—Individuals with short forelimbs arise from ones that lost these forelimbs earlier in ontogeny. I encountered individuals with short forelimbs far less frequently than ones missing forelimbs (24 vs. 110; 3.8% vs. 18.1%; $G_1 = 127.08$, $P < 0.0001$, goodness of fit, $n = 633$).

Carapace width did not differ significantly with the presence of short forelimbs (Table 2). Neither mass (Table 2) nor mass corrected for the short limbs (Table 2) differed significantly, the result of a single anomalously large individual regenerating two forelimbs. Of the other variables measured (date of death carapace width, year, site of collection), none was significant (Table 2).

I obtained one penultimate male with a short forelimb (1 of 52 = 1.9%, not differing significantly in frequency from adult males ($G_1 = 0.50$, $P > 0.3$). I have also reared additional penultimate males in the laboratory that lacked a forelimb and subsequently molted into the adult stage with half-length forelimbs similar to those seen on the 24 adult males reported here. I only obtained occasional penultimate and adult females with short forelimbs in much larger samples (penultimate: 1 of 336 = 0.8%; adult: 2 of 862 = 0.2%). These frequencies fall significantly below those of the adult males ($G_1 = 13.85$, $P < 0.001$; $G_1 = 28.84$, $P < 0.001$, respectively). I found no early instars with missing forelimbs (0 of 227).

Survival in field and laboratory.—Many confined male *Misumena* lived for periods considerably longer than the

Table 2.—Characteristics of adult male *Misumena vatia* with 0–2 short forelimbs (mean ± SD), and results of ANOVAs. Superscripts 1–4 as in Table 1; [5] After removal of an anomalously large individual of 8.9 mg, mass = 2.93 ± 0.847 mg and mass corrected = 4.08 ± 0.777 mg.

Variable				*F*	*df*	*P*
Number of short forelimbs (*n*)	0 (491)	1 (17)	2 (7)			
Carapace (mm)	1.47±0.149	1.45±0.140	1.46±0.178	0.23	1,513	0.63
Mass (mg)	4.58±1.540	4.06±1.134	4.21±2.222 [5]	1.71	1,513	0.19
Mass corrected[1]	4.58±1.540	4.32±1.208	4.77±2.516 [5]	0.03	1,513	0.87
Date of capture[2]	172.1±10.66	175.1±12.98	170.9±9.10	0.2	1,513	0.65
Date of death[2]	203.4±18.11	204.1±18.54	215.1±28.27	0.63	1,262	0.43
Year[3]	4.5±4.20	4.9±4.40	2.3±3.90	0.73	1,513	0.39
Collection site[4]	2.7±1.57	3.4±1.52	2.4±1.81	0.27	1,513	0.60

maximum dates I have recorded in the field. During one year that I weekly censused *Misumena* in all flowers at eight sites in the vicinity of the Darling Center (total = 0.72 ha), I failed to record adult males after 28 July, a pattern consistent with less systematic observations made over the 2000–2012 period, in which I have rarely found adult males in the field after that date. However, I have frequently maintained males in the laboratory until late August and early September, and 74.6% of the males retained in this study ($n = 303$) survived past 28 July. I did not find significant differences in longevity in the laboratory between intact individuals and those that had lost forelimbs (ANOVA: $F_{1,300} = 0.11$, $P = 0.742$).

DISCUSSION

Possible sources of forelimb loss.—Male *Misumena* lose forelimbs at a highly significantly greater rate than either penultimate or adult females. Forelimb loss of penultimate males is more comparable to that of the adult males than of females or juveniles and provides insight into the frequency of loss among the adult males. Unfortunately, the sample of penultimate males is relatively small, since virtually all of the males since 2002 have molted into the adult stage before our field season begins in early June, part of a pervasive shift in the phenology of several species monitored since 1995 in the main study area (D.H. Morse, unpubl. data).

Adult males engage in aggressive interactions when near each other, but penultimate males, which have a comparable rate of forelimb loss, generally do not undertake high-level interactions (Holdsworth & Morse 2000). A relatively low rate of forelimb loss of adult males even in staged encounters between males at the sites of late-stage penultimate females (3 in 90 encounters, 3.3%: Hu & Morse 2004) and in mating experiments (Morse 2010) makes the putative role of adult male aggression unlikely as a sole or principal source of forelimb loss. Opportunities for these interactions are relatively infrequent in the field, much lower than in the closely related *Misumenoides formosipes* (Walckenaer, 1837) (Dodson & Beck 1993; Dodson et al. 2015). The similar size (carapace width) of individuals with different numbers of missing forelimbs is also inconsistent with aggressive interactions playing the major role in limb loss. Otherwise, one might expect large dominant males to produce higher limb losses in combat than smaller ones, because large individuals initiate attacks more frequently than smaller ones, a pattern seen in other species as well (Jakob 1994; Hu & Morse 2004). Hence, the high frequency of missing limbs in adult males is unlikely to result solely from male aggression.

Mating is a dangerous experience for male spiders, with some species even dying after mating, including instances in which they are killed by their mates (Andrade 1996; Foellmer & Fairbairn 2003; Schneider et al. 2006). Mating takes a less frequent toll in *Misumena*, but aggressive females do regularly capture courting males. However, I have found that in virtually all instances the females strike directly at the body, and the male either escapes intact or is captured and killed (Morse & Hu 2004; Morse 2010). Predators vary in their tendency to strike at a spider's body or limbs (Formanowicz 1990). Thus, male-female interactions also appear unlikely to account for a major part of the observed limb loss.

Male forelimbs are especially long and slender and thus potentially vulnerable to loss. Although not as long as those of adults (Morse 2007), penultimate forelimbs are nevertheless extremely long and slender relative to those of females (Morse 2007) and are thus potentially vulnerable to predators or to entanglement in the vegetation (Maginnis 2006b). On the basis of occasional observations made while collecting males in the field, I predicted that the long, gracile form of male forelimbs would enhance their vulnerability to entanglement in the vegetation (petiole-stem interstices, etc.), especially if suddenly attacked. Analogously, web-building spiders may autotomize limbs if tangled in webs (Johnson & Jakob 1999).

Although spiders may experience difficulty in extracting their limbs from their old molt (Maginnis 2006a; Foelix 2011), male *Misumena* molt successfully in the laboratory, as long as I maintain adequate humidity. Problems of low humidity are probably even less likely to occur in the field. Thus, this potential problem appears unlikely to account for a major part of forelimb loss of the males. The frequency of missing forelimbs in the penultimate males appears adequate to suggest that many of the adults missing forelimbs suffered the loss earlier in ontogeny. Pasquet et al. (2011) and others have reported losses of less than 1% in penultimate males of other species.

Short forelimbs.—Adult males with short forelimbs experienced an even greater disadvantage under natural conditions than those completely lacking limbs, in that they performed a number of movements more slowly than even those missing limbs, and most of them also lost condition (Lutzy & Morse 2008). Some spiders held partially regrown limbs away from the body, which therefore did not contribute to locomotion (Vollrath 1990) or prey capture (Wrinn & Uetz 2008). The spiders also expend considerable energy growing these new limbs (Maginnis 2006a; Bely & Nyberg 2009). Thus, it appears that these males would profit from losing their regenerative ability, especially in the later instars. Indeed, one could imagine such selective pressures having driven the loss of regeneration in groups that have lost this ability. Losses of the ability to regenerate limbs have occurred many times among groups that exhibit autotomy and appear related to conditions routinely experienced in the field (Bely & Nyberg 2009).

Since regeneration only produces external limbs at molts, none of the small replacement forelimbs result from losses in the adult stage. Most likely the adult males with partially regenerating forelimbs lost their forelimbs early in their penultimate stage. In some species of spiders, regeneration will occur if limb loss takes place during the first quarter of an individual's penultimate instar (Foelix 2011, citing Bonnet 1930). This prediction is consistent with the statistically similar level of forelimb loss seen in adult and penultimate males.

Male survival.—The difference between apparent adult male survival in the field and laboratory suggests that they seldom reach their maximum potential life span in the field. In the field, males spend most of their adult lives vigorously searching for females (LeGrand & Morse 2000). Although adult males do feed (vs. males of many spiders: Foelix 2011) and largely retain their weight over time, their high level of activity (Holdsworth & Morse 2000) presumably takes its toll. Male spiders regularly die before their females (e.g., Dodson & Schwaab 2001). The similar survival in the laboratory between

intact individuals and those lacking forelimbs was initially surprising, because individuals lacking forelimbs were significantly lighter (after correetion for missing forelimbs) and in poorer condition than intact individuals when captured. This result is probably a consequence of the easily captured food in the laboratory, which permitted feeding to satiation. Thus, the poor condition of individuals lacking one or more forelimbs in the field may result from decreased suceess in prey eapture under complex conditions (Brueseke et al. 2001), as well as compromised locomotor capabilities (Lutzy & Morse 2008). The laboratory-retained individuals lacking one or more forelimbs would presumably have performed poorly in a set of locomotor tasks eomparable to those they would experience in the field. In fact, field-captured individuals could not travel on lines as rapidly as intact ones (Lutzy & Morse 2008). If they followed the pattern observed in intact individuals (Lutzy & Morse 2008; Morse 2014), they probably would score poorly in other locomotor activities (running, climbing) as well as in line-crossing. Thus, forelimb loss results in eompromised body condition and performance.

Regeneration and forelimb loss.—Bely & Nyberg (2009) identify the question of why regeneration persists where it appears to be irrelevant as a major unaddressed question in regeneration studies. Although regeneration of forelimbs appears disadvantageous to penultimate and adult male *Misumena*, it may be advantageous in early instars. However, the earlier *Misumena* instars exhibit significantly lower frequencies of forelimb loss than the penultimate and adult males, which would suggest that selection could not operate as strongly as on the older males. Regeneration might also be advantageous for the females; however, they have strikingly lower frequencies of lost or short forelimbs than the males, making the explanation problematic.

Thus, the results leave a major unanswered question: why are male and female forelimb losses so different? Results presented here and in the literature suggest that this difference involves a conflict between robustness and speed: the difference between the ability of females to manipulate large prey and the ability of males to move quickly when searching for females.

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Female feeding history impacts gonad development and reproductive timing in the wolf spider *Schizocosa ocreata* (Hentz, 1844)

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Abstract. In mating systems that include semelparous reproduction and/or scramble competition, synchronous maturation of the sexes is vital for success. However, food limitation may alter the onset of maturation or the overall quality of the mature individuals and affect reproductive success. We examined the role of feeding history (well-fed vs. long-term deprivation) on female reproductive timing and its correlation with temporal patterns of receptivity behavior in the wolf spider *Schizocosa ocreata* (Hentz, 1844). We found that feeding history influenced developmental time and delayed maturation in long-term food-limited females. There was no significant difference in relative condition between treatments, yet well-fed females showed higher rates of receptivity. Peak receptivity behavior was correlated with the estimated overall mass of female ovaries/eggs, with females that possess larger ovaries and eggs showing more receptive behavior. This supports the hypothesis that while a food-limited female may attain maturity, the limiting factor underlying reproductive success is gonad maturation.

Keywords: Fecundity, diet, receptivity, egg development

Understanding the interactions between nutrition and the development of reproductive anatomy is important when addressing mate choice, sexual selection, and sexual conflict (Arnqvist & Rowe 2005; Uetz & Norton 2007). For semelparous organisms with well-defined seasonal reproduction, many factors may impact the onset and maintenance of receptivity and courtship within a species (Lehrman 1965; Barth & Lester 1973). This ontogeny may be linked to the natural seasonality of the environment and impose intrinsic control over the ability to acquire food and allocate sufficient energy to reproductive efforts. Throughout the animal kingdom, sexually reproducing species are constrained by the maturation of reproductive structures and the initiation of sex specifie behaviors, as these behaviors are often associated with hormones released by the developing gonads (Lehrman 1965; Barth & Lester 1973; Ringo 1996).

Maintenance of physiological condition and gametes is also important to reproductive success, and conflict exists between mating age, egg maturity and egg maintenance (Moore et al. 2007). Research has demonstrated links between female reproductive state, egg maturity, female receptivity and aggression in both solitary and subsocial spiders and some insects (Trabalon et al. 1988,1992; Wilgers & Hebets 2012). In spiders, females have been shown to produce different levels of hormones in relation to egg maturation, which contribute to female tolerance of conspecifics (Trabalon et al. 1988,1992). Even so, no research on spiders has yet examined the condition-dependent nature of female ovary development and its relationship to behavior, or the temporal consequenees of diet on reproductive physiology. There is, however, evidence for compensatory development in spiders, based on recovery of body size and mass after nutritional stress (Jespersen & Toft 2003). Additional support suggests that during times of stress (i.e., food deprivation) there should be compromise in physiological processes (Gustafsson et al. 1994; MeNamara & Houston 1996). It is well-established that diet has direet consequenees for reproduetive investment of females (Enders 1976; Simpson 1995; Parker & Begon 1996; Toft & Wise 1999; Kreiter & Wise 2001). Thus, while much speculation exists about egg development/investment in spiders (Foelix 1996), little else is known about the direct effects of diet on gonad development. By examining the interactions between diet and gonad development, we will better be able to see how physiological limitations imposed by long-term diet restrictions impact mate choice decisions of females.

For many spider taxa, there are age-related differences in mate choice and female receptivity, frequently accompanied by differential male courtship investment (Norton & Uetz 2005; Uetz & Norton 2007; Wilgers & Hebets 2012; Rundus et al. 2015). In the wolf spider *Schizocosa ocreata* (Hentz, 1844), females have a predictable receptivity cycle after maturity, in which they are initially highly resistant to male advances (Week 1) followed by a level of high receptivity (Week 3) and a return to resistance (Week 5 and later); high levels of resistance are also demonstrated after mating (Norton & Uetz 2005; Uetz & Norton 2007). Uetz & Norton (2007) suggested this pattern might be related to potential availability of males, as phenology of maturation in this species is typically asynchronous (i.e., males tend to mature before females, leading to a male-biased sex ratio early in the breeding season). However, long-term observations (Uetz & Roberts, unpubl.) have shown that maturation synchrony does vary from year-to-year with weather factors. Additional research has demonstrated that this cycle is also condition dependent (Moskalik & Uetz 2011), suggesting the possibility that concomitant faetors sueh as gonad development might be driving receptivity. The analysis of egg maturity during phases of this behavioral cycle may therefore shed light on what may be driving female reeeptive and aggressive behavior.

Here, we tested two hypotheses: (1) diet will affect development of and total investment in reproductive structures of female spiders for both penultimate (follicle number, size) and adult *S. ocreata* (fecundity, egg size, volume, total clutch volume) and (2) female ovary and egg maturation in well-fed spiders will coincide with the previously described

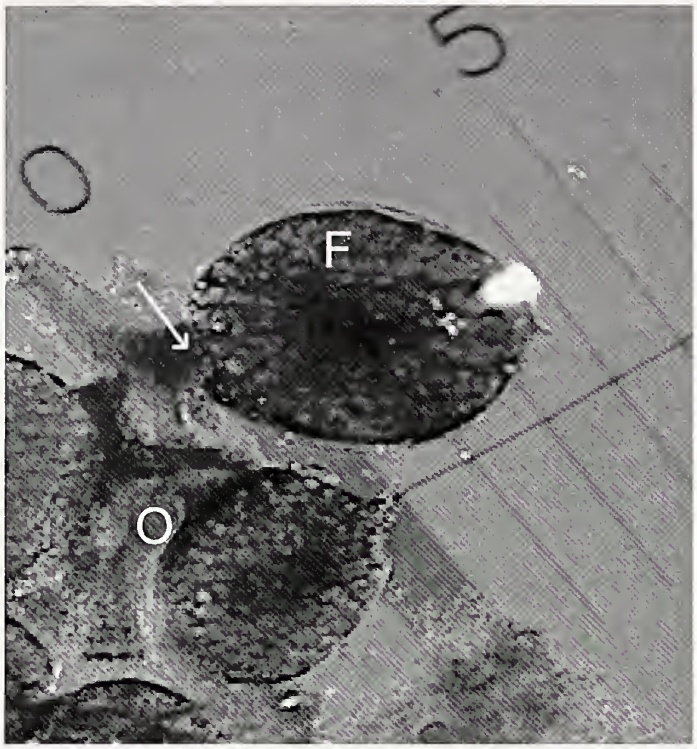

Figure 1.—Image of ovulated follicles (oocytes) from a well-fed ultimate female 21 days mature. F shows an individual follicle, the arrow indicates pedicle/funiculus, and O indicates a fragment of the ovary.

Figure 2.—Biramous ovary with follicular development from well-fed penultimate female. F indicates follicle clusters and U the oviduct/uterine tube.

receptivity curve (Uetz & Norton 2007) and influence behavior in a predietable manner. We predicted that if diet impacts reproductive structure development, then the food-limited group should demonstrate smaller average gonad size and reduced numbers of ovulated follicles and/or mature ova compared to the well-fed group. Also, if female ovary development impacts receptivity, a relationship between egg maturation (size) and previously observed temporal patterns of receptivity will be seen, with female peak receptivity coinciding with the time that eggs become larger because of the accumulation of yolk.

METHODS

Spider maintenance.—Hatchling spiders were obtained from field collected females with egg sacs and maintained in deli-style containers (9 cm height x 5 cm width). Spiders were held in the laboratory on a 14:10 light:dark cycle at 23°C and randomly assigned to well-fed or food-limited dietary protocols (Uetz et al. 2002; Balfour et al. 2003). Both treatments received gut-loaded crickets (*Acheta domesticus*) with well-fed individuals receiving 100% of their mass twice per week and food-limited individuals receiving 50% of their body mass once per week. Diet implementation and growth were followed from hatching to adulthood. Individuals were then randomly assigned to assay groups (penultimate; week 1 post ultimate molt, hereafter mature week 1; week 3 post ultimate molt, hereafter mature week 3).

Assessment of female growth.—We assessed all females and compared mass, cephalothorax width (CW) and developmental time (total days to maturity and number of instars) between starvation and well-fed treatment groups with a one-way ANOVA. To assess female body condition between diet treatments, we used an ANCOVA of weight x treatment, and adult cephalothorax width (CW) as the covariate (Marshall et al. 2000; Garcia-Berthou 2001; Schulte-Hostedde et al. 2005).

Assessment of female receptivity.—We assessed female receptivity with behavioral assays at three designated times (penultimate, mature week 1, mature week 3) matching those of earlier studies of female behavior (Uetz & Norton 2007). Females were placed with well-fed, lab-reared males in an arena for 5 minutes and observed for receptive behavior and male mounting behavior. The 5-minute duration has been shown to be an effective time frame to determine female choice (Moskalik & Uetz 2011). Males were mature for three to five weeks and in apparently good condition. To control for male variation, if no mating occurred, the first male was removed and a second male was placed in the arena and allowed to court for another 5 minutes. If mounting occurred, the pair were immediately separated and the female was euthanized under CO_2, preserved in AAF fixative (10 ml concentrated formalin: 5 ml glacial acetic acid: 75 ml 96% ethyl alcohol: 10 ml distilled water) and dissected to examine egg development.

To examine the effect of diet treatment on spider development, we compared well-fed and food-limited treatment females (n = 75 and 46, respectively) using one-way ANOVA. The effect of treatment on behavior was assessed by using a Chi square comparison of proportion of receptive

Table 1.—Female development. One-way ANOVA analyses of female development. Bold *P* value indicate significant differences between diet treatments

Source	df	F ratio	*P*
Mature weight	1	8.534	**0.004**
Mature cephalothorax	1	7.871	**0.006**
Mean instar length	1	0.024	0.877
No. of molts	1	8.762	**0.004**
Total development duration	1	6.513	**0.012**

adult females at two points in time after maturity (Week 1 and Week 3).

Images of eggs and oocytes were taken with an Olympus digital camera (Figs. 1, 2). Measurements of egg development included total egg number and mean egg diameter. Mean diameter was generated by measuring the width of 10 eggs at random or 10% of the clutch, whichever was greater. Using the standard equation for the volume of a sphere, we estimated the volume of the eggs using the mean diameter. A measurement representing total investment (total clutch volume) was derived by multiplying the mean volume by the total number of eggs. Egg number and total estimated clutch volume were analyzed with a two-way ANOVA, with treatment (starved vs. well-fed) and female age (penultimate, mature week 1, mature week 3) as factors.

RESULTS

Spider development.—Stadia did not differ significantly between treatments, but number of instars, total duration of development, cephalothorax width, and adult mass varied significantly with diet (Table 1). Tukey post hoc examinations revealed that mean stadia did not differ significantly between treatments (Well-Fed: 27 ± 4.5 vs. Starved: 27 ± 4.8 days; $F_{1,91} = 0.024$, $P = 0.89$) but there was a significant effect of diet on number of instars, with well-fed females requiring fewer molts to attain maturity (Well-Fed: 6.68 ± 0.14 vs. Starved: 7.32 ± 0.17 molts respectively; $F_{1,91} = 8.76$, $P = 0.004$). Total developmental time varied significantly with well-fed females maturing fastest (Well-Fed: 179 ± 4.9 vs. Starved:196 ± 4.5 days: $F_{1,91} = 6.51$, $P = 0.012$). Well-fed females were also larger and heavier than the long-term starvation groups (Table 1). Results of female body condition indicate that relative condition (i.e., mass scaled to body size) did not vary by treatment when cephalothorax was accounted for ($F_{1,49} = 0.94$, $P = 0.35$).

Behavior.—In week 1, females within each treatment were equally likely to be receptive to males ($n = 20$, $X^2 = 0.02$, $P = 0.89$; <20% of females). However during week 3, treatment significantly impacted the proportions of females receptive to males ($n = 20$, $X^2 = 6.54$, $P = 0.011$) with 60% of well-fed females showing receptivity, versus 20% of limited females becoming receptive.

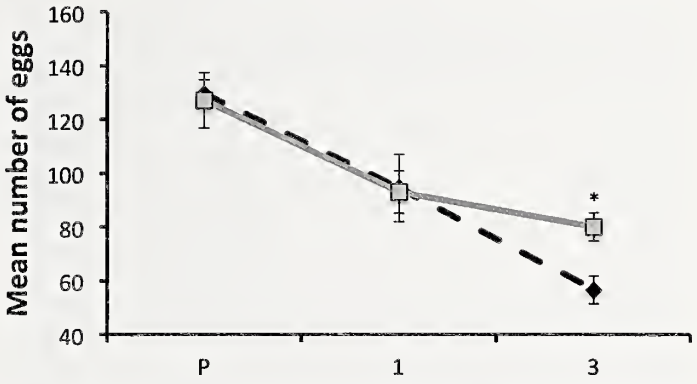

Figure 3.—Graph showing the decline and divergence of mean egg number ± standard error between diet treatments. The gray solid line represents the well-fed group and the dashed line represents food-limited group. * Indicates a significant difference.

Comparisons of egg development.—Egg number (Table 2) per female was normally distributed and was analyzed with a multifactor ANOVA, revealing whole model significance, a significant difference between weeks, and a significant interaction between diet and week (Table 3). Post hoc analyses revealed that egg numbers declined uniformly between treatments but by week 3, low diet treatments were significantly lower than well-fed (Table 2, Fig. 3). Egg diameter was not normally distributed (Shapiro-Wilks W = 0.92, $P = 0.012$) and was examined for outliers. Grubbs outlier test (JMP 8) confirmed two outliers that were removed and the subsequent distribution was then normal (Shapiro-Wilks W = 0.95, $P = 0.19$). The same multifactor ANOVA was applied and indicated whole model significance and demonstrated that week and diet both impacted egg diameter but there was no interaction (Table 4). As expected, well-fed females had larger eggs than food limited females (Tables 2, 4) and egg diameter significantly increased with time (Tables 2, 4). Penultimate females had the smallest diameter with eggs growing successively larger each week (0.13 ± 0.006 mm vs. 0.14 ± 0.003 mm vs. 0.16 ± 0.003 mm: penultimate, weeks 1 and 3 respectively). The total clutch investment showed significant right skew (Shapiro-Wilks W = 0.80, $P < 0.0001$) and was Box Cox Y transformed for best fit (Shapiro-Wilks W = 96, $P = 0.19$; JMP 8). The multifactor ANOVA showed whole model significance and that diet and a diet x week interaction

Table 2.—Egg developmental characteristics based on treatment.

Treatment	Age	No. of egg follicles	Follicle diameter (mean, mm)	Follicle diameter (range, mm)	Estimated clutch volume (mm^3, investment)
Well-fed	*Penultimate*	127	0.133	0.125–0.148	0.165
	Week 1	86	0.152	0.137–0.179	0.168
	Week 3	81	0.189	0.157–0.233	0.305
Food-limited	*Penultimate*	123	0.130	0.122–0.138	0.114
	Week 1	91	0.121	0.115–0.127	0.105
	Week 3	51	0.151	0.145–0.157	0.091

Table 3.—Two-way ANOVA analyses of mean number of eggs. Bold *P* values indicate significant differences.

Source	df	F ratio	*P*
Whole model	5	11.79	**<0.0001**
Treatment	1	2.1011	0.16
Week	2	24.6155	**<0.0001**
Treatment*Week	2	3.9543	**0.03**

Table 5.—Two-way ANOVA analyses of mean total clutch volume. Bold *P* values indicate significant differences

Source	df	F Ratio	*P*
Whole model	5	9.16	**<0.0001**
Treatment	1	14.99	**0.0006**
Week	2	1.08	0.35
Treatment*Week	2	4.09	**0.03**

significantly impacted total investment (Table 5). Tukey post hoe analyses revealed that initial cluteh volume was equal in both groups and there was a divergence for both groups in week 1; in week 3, the divergence inereased (Fig. 3).

DISCUSSION

Our results support previous findings that females who were fed more grew faster and produced more eggs. As a consequence, development and maintenance of follicles and eggs was compromised in starvation treatments. The impacts, however, were not significant until later in maturity (week 3). This suggests that female feeding history can impact egg maintenance, which could potentially affect receptivity behavior and subsequent mating or mate choice decisions. As predicted, follicular development mirrored the behavioral tendencies first observed by Uetz & Norton (2007). We observed that a redueed female feeding history significantly reduced the likelihood of female receptivity over time. From these results, we can infer a correlation between female age, diet, ovary/follicle development and receptivity. The initial results suggest that females invest equal amounts of resources into follicles regardless of treatment during their penultimate stage until the onset of adulthood. Then as maturity and adulthood ensue, females diverge in their ability to 1) maintain overall egg numbers and 2) invest in egg nutrition that would subsequently support the post-embryonic spiderling.

This research also highlights the impact that diet has on spider developmental plasticity, as *S. ocreata* demonstrated marked plasticity in developmental time. Well-fed females were able to mature in fewer instars and still attain a population mean adult size. On the other hand, food-limited females were delayed in maturation by the addition of one or two more instars, thus passing through 9–10 post emergent instars, whieh has been previously reported for this speeies (Amaya & Klawinski 1996). These late maturing females were significantly smaller than the rest of the laboratory population. However, an ANCOVA based on mass scaled to body size revealed no differences. This finding raises questions regarding the sensitivity of ANCOVA vs. a ratio or residual body condition index (BCI) when examining development and plasticity within a population subjected to varying food abundance.

Table 4.—Two-way ANOVA analyses of mean egg diameter. Bold *P* values indicate significant differences.

Source	df	F ratio	*P*
Whole model	5	12.61	**<0.0001**
Treatment	1	11.26	**0.002**
Week	2	14.97	**<0.0001**
Treatment*Week	2	1.30	0.29

Previous research on spider ovary development has described the developmental stages eggs go through as they mature and how these correlate with female ecdysteroid levels in the spiders *Coelotes terrestris* (Wider, 1834) and *Tegenaria domestica* (Clerck, 1757) (Trabalon et al. 1988, 1992). There were several distinct differences between these results and our wolf spider population. Seemingly, the development of follicles in these agelenid spiders oecurs after maturation, whereas in our lycosid speeies it begins during the penultimate stage. Additionally, the growth of the agelenid spiders was very robust, with each individual requiring a fixed number of instars to reach adulthood. Our lycosid spiders, even in the absence of diet variation, showed some plasticity in maturation time and could mature earlier than the expected population maxima (8^{th} instar post emergence).

Research presented here demonstrates the importanee of female diet and how it affects growth, behavior, reproduction and fecundity of female *S. ocreata* wolf spiders. While growth shows plasticity, certain other developmental aspects do not. Female follicular ovulation seems to be fixed with respect to the number of initial oocytes generated. There are clearly more oocytes ovulated than can be supported by these females, as even the well-fed group had an intrinsic "rate of decay" within the first week of maturity. The loss of eggs may be a timing event that eventually signals the maximum clutch amount for the female, thus representing an "internal clock". This observation generates many interesting avenues of inquiry that deserve future attention. Female behavior appears to be regulated by feeding; perhaps food limited females have a

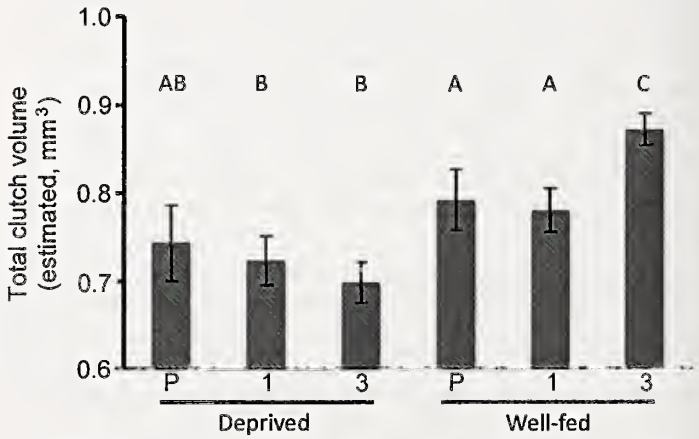

Figure 4.—Estimated total volume of clutch in mm^3 (bars indicate standard error). Letters indicate significant differences within and between treatments [*P* = penultimate instar; 1, 3 indicate weeks post-maturity) based on Tukey post hoc test.

continuous rate of egg loss and ideally benefit from cannibalism, not mating, in order to support fecundity. However once egg loss slows or stops, mating should ensue, regardless of feeding, as maximal fecundity has been reached for that individual. Future studies should address potential trade-offs and physiological relationships between fecundity, survival and maturation timing.

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Influence of predator cues on terminal investment in courtship by male *Schizocosa ocreata* (Hentz, 1844) wolf spiders (Araneae: Lycosidae)

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Abstract. Sexual signals play a critical role in mate attraction, but fitness benefits of signal production depend on a number of external and internal influences. Sexual signaling can be energetically expensive, and has potential to attract unwanted attention from predators. Male brushlegged wolf spiders, *Schizocosa ocreata* (Hentz, 1844) (Araneae: Lycosidae), actively signal to females in the leaf litter habitat during their spring breeding season, but face a tradeoff between current and future reproduction as the season progresses. The terminal investment hypothesis predicts that with fewer available females, increasing risk of predation, and stronger influence of senescence as the season progresses, males should take greater risks to secure mating. We explored this idea by exposing males of increasing ages to female cues alone or female cues combined with predator cues. We found little or no direct evidence to support the terminal investment hypothesis in this species, in that males across all ages essentially ceased active courtship in the presence of predator cues, that is, there was no age related increase in courtship investment in the presence of predator cues. However, we found distinct evidence of senescence in males based on age-related changes in behavior, which has not previously been directly explored in this species. While males maintained similar levels of active courtship across all age classes (in the absence of predator cues), older males increased their relative investment in maintenance behaviors (grooming) and decreased non-courtship display behaviors such as tapping and leg raises. These findings suggest that studies of male behavior in this species should be carefully designed to control for age-related variation in behavioral response.

Keywords: Senescence, predation, age effects, chemical cues, context dependence

Sexual signaling is known to be critical for mate attraction in many species. Individuals produce signals that have been shaped over evolutionary time to maximize transmission, reception, and receiver response (Andersson 1994; Johnstone 1996; Rowe 1999; Bradbury & Vehrencamp 2011). Male sexual signals are often elaborate and conspicuous, potentially indicating male quality to females through size and/or symmetry of traits or degree of courtship vigor (Clutton-Brock & Albon 1979; Parker 1983; Kodrick-Brown & Brown 1984; Hebets & Uetz 1999; Byers et al. 2010). However, sexual signals do not evolve in a vacuum and the fitness benefits associated with signaling are contingent upon both external (ecological/environmental) and internal (physiological) factors. Many studies have shown that male traits favored by females through mate attraction impose energetic costs and/or increased the risk of predation on males (Andersson 1986; Magnhagen 1991; Zuk & Kolluru 1998; Roberts et al. 2007; Cady et al. 2011), but far fewer studies have investigated the combined effects of physiological condition, such as age-related performance declines (i.e., senescence), and external influences (e.g., predator cues) on courtship behavior.

Selection should favor males who respond to internal and external influences in a way that maximizes potential fitness benefits associated with signaling (Bradbury & Vehrencamp 2011; Reichard & Anderson 2015). This is especially true for males that face a declining chance of reproduction due to senescence and/or increasing predation. The terminal investment hypothesis suggests that males who face a tradeoff between current and future reproduction, especially where chances of future reproduction are small, should increasingly invest effort in high risk/high reward behaviors like active, complex signaling and courtship (Clutton-Brock 1984; Part et al. 1992). Such an investment might increase mortality and/or the influence of senescence (Bonduriansky et al. 2008), but would raise the chances of successful reproduction even when obstacles to reproduction are ever increasing (Clutton-Brock 1984; Bonduriansky et al. 2008).

The brushlegged wolf spider, *Schizocosa ocreata* (Hentz, 1844), has been a rewarding model for the study of sexual signaling and mate choice (Uetz & Roberts 2002; Hebets & Papaj 2005), and can be used as a model for investigating issues of behavioral plasticity and context-dependent signaling (Hebets 2011; Clark et al. 2012). *Schizocosa ocreata* is a common ground-dwelling wolf spider abundant in leaf litter of eastern deciduous forests of North America (Dondale & Redner 1990). Females are cryptic and relatively sedentary within the leaf-litter environment, while males traverse the forest floor and actively seek and court hidden females by displaying complex, multimodal signals (Aspey 1976; Cady 1983; Uetz & Roberts 2002; Uetz et al. 2013). Females select males based on size and symmetry of morphological characters (tufts) as well as aspects of courtship vigor (Uetz & Roberts 2002; Hebets & Papaj 2005; Byers et al. 2010). Female receptivity to male courtship increases until females reach approximately three weeks post adult molt, after which receptivity begins a steady decline with advancing age (Uetz & Norton 2007). Males will mate multiple times given the opportunity in this scramble-competition polygyny system (Norton & Uetz 2005; Uetz & Norton 2007), but females typically mate only onee after which they become highly

aggressive toward further mating attempts, attacking and often cannibalizing the male (Uetz & Norton 2007).

The silk draglines and associated chemical cues deposited by females as they move through the environment play a critical role in elieiting male eourtship. The cues of a female eonspecific elicit male courtship responses even in the absence of the female (Stratton & Uetz 1981), and provide valuable information to males ineluding species identity, female age, and mating status (Roberts & Uetz 2004a, b, 2005). Males can also detect and discriminate heterospecifie, potentially predatory spider species, and aggressive, mated female conspecifics by their silk and chemieal cues, and have shown a decreased courtship response to potentially dangerous congeners, especially predators (i.e., *Tigrosa* spp., see Persons et al. 2002; Roberts & Uetz 2004b; Fowler-Finn & Hebets 2011). The breeding season of *Schizocosa ocreata* occurs for a relatively brief, 5–8 week period in the spring (May/June), and the relative proportion of available, unmated females decreases while the number of potentially eannibalistic, mated females inereases (Roberts unpubl.). Males, therefore, have a decreasing chance of mating and increasing chance of being eaten by aggressive females or heterospeeifie predators as the season progresses.

Here we investigate the terminal investment hypothesis for male *S. ocreata* by exploring the interaction between physiological condition (age/senescence) and suppression of courtship indueed by environmental predator cues. If the terminal investment hypothesis is valid in this species, then males should exhibit plastieity in their courtship behavior in response to external and internal conditions. Males decrease investment in conspicuous courtship behavior in the presence of predator cues in general (Roberts & Uetz 2004b; Fowler-Finn & Hebets 2011), but if males suffer reduced reproductive potential as they age, older age classes will be more likely to engage in risky courtship behavior, that is, courtship in the presence of predator cues. We compared the courtship behavior of males from four different age groups exposed to female cues alone or to combined female and predator cue treatments to determine whether males exhibit a plastic courtship response to either ecological or physiological factors.

METHODS

Spider collection and maintenance.—Juvenile *Schizocosa ocreata* were collected from The Dawes Arboretum, Licking County, Ohio, USA (39.97849°N, 82.41614°W) in late April 2010. Female sub-adult and adult *Tigrosa helluo* (Walckenaer, 1837) were collected from Waterman Farm at The Ohio State University, Franklin County, Ohio, USA (40.01220°N, 83.03937°W) in October 2009 and May 2010. Only female *T. helluo* were used in experiments, as females of this species are considerably larger and generally more likely to attack prey than males or juveniles (Walker & Rypstra 2002), and are known to readily accept *Schizocosa* as prey (Roberts, personal observation). *Schizocosa* were housed individually in plastic containers (540 ml, round), with ~20 mm moistened peat moss as a substrate and *ad libitum* water source, and *Tigrosa* were housed similarly in larger containers (950 ml) with more substrate (~50 mm) to allow burrowing. All individuals were maintained at room temperature (22–25°C), stable humidity, and a 13:11h light:dark cycle to simulate spring lighting conditions. *Tigrosa helluo* were fed a bitypic diet once a week that included one to two adult crickets and one to two mealworms. *Schizocosa ocreata* were fed twice weekly with three to four fruit flies (*Drosophila melanogaster*) or two to three 1-week-old cricket nymphs (*Acheta domesticus*) as appropriate for their size. All *S. ocreata* were cheeked daily for ecdysis to determine date of maturation for tracking adult age following the ultimate molt.

Silk collection and substrate preparation.—Wolf spiders deposit silk and ehemical eues as they traverse their environment, and female eues, even in the absence of females themselves, are known to induce males to court (Stratton & Uetz 1981; Roberts & Uetz 2005; Foelix 2011). Further, silk and chemical cues of *Tigrosa* spp. are known to elicit anti-predator behaviors in this and other wolf spider species (Roberts & Uetz 2004b; Bell et al. 2006; Fowler-Finn & Hebets 2011). In order to induce *S. ocreata* male courtship and/or anti-predator responses, we colleeted silk and associated cues from conspecific females, and from predatory female *T. helluo*. Prior to each trial, we placed an individual female *S. ocreata* on a clean sheet of filter paper (Fisherbrand, 90 mm diameter, round) in an opaque plastie container (90 mm diameter) and using a small brush, gently induced her to make 50 laps around the outside of the filter paper to standardize the amount of cue material deposited. Female conspecifics used for cue deposition were unmated and ranged in age from two to four weeks post-ultimate molt (period of peak receptivity, see Uetz & Norton 2007). Filter papers used in the predator trials were first laden with conspecific female cues as above, after which we placed individual *T. helluo* on each filter paper and induced them to make 50 laps in the same manner as *S. ocreata* females, depositing their cues on top of the *S. ocreata* cues. Preliminary experiments showed no difference in male signaling behavior resulting from order of cue deposition in predator trials. Individual spiders were used only once for silk deposition and no spider was fed within 24 hours of trials, to both standardize hunger and reduce fecal contamination of cues. All trials occurred within 10 minutes of completing the silk deposition stage.

Experimental design.—To test the hypothesis that differences in male age are correlated with differences in courtship behavior in the presence of predator cues, we conducted a two-way MANOVA design experiment with male age (one to four weeks of maturity) and predator cues (present/absent) as factors, individuals as replicates, and behaviors (Table 1) as multiple dependent variables. The cohort of males available for this study all matured within a five day period in order to synchronize age effects and the timing of trials as closely as possible. We selected 90 male *S. ocreata* from the lab population as they molted to maturity and randomly assigned each to one of the eight, age-by-predator cue treatment groups (final sample sizes were approximately 11 per treatment group). We used each male only once within 48 hrs of reaching the appropriate age post adult molt such that males "one week old" were six to eight days post maturity when used in experiments, males two weeks old were 13 to 15 days post maturity, etc.

We conducted behavioral assay trials in clear plastic arenas (250 × 100 x 100 mm) where we placed filter paper disks

Table 1.—Ethogram of male *Schizocosa ocreata* behaviors (adapted from Stratton and Uetz 1986; Delaney et al. 2007).

Behavior	Description
Jerky Tap	Active, visual and seismic courtship where the male locomotes with rapid jerky movements while tapping the forelegs, and occasionally the ventral body surface, on the substrate. Seismic signals in the form of percussion and stridulation are also produced.
Tap	Sometimes called double tap, one or both forelegs actively tapped on the substrate.
Leg Raise	Also called "arch" and/or "wave", one or both forelegs is raised above parallel to the substrate then lowered without striking the substrate.
Chemoexplore	Exploratory behavior where the anteriolateral palp surfaces are rubbed on the substrate while slowly locomoting.
Grooming	The legs or pedipalps are drawn through the chelicerae, or lateral pairs of legs are brushed together rapidly.
Locomotion	Walking, includes wall climbing.
Stationary	Motionless.

containing cues of female conspecifics, and predators as appropriate, silk side up on the bottom of the arena immediately prior to the onset of each trial. We then carefully deposited males into the arena from above and video-recorded their response to cues for 300s. Following each trial, we removed and discarded the cue disks, cleaned the arena using 70% ethanol and a Kimwipe® to remove any residual chemical or silk cues, and allowed the arena to air dry. All recorded trials were later scored for total duration (s) and frequency (number/300s) of male courtship (Jerky Tap), display (Tap and Leg Raise), exploratory (Chemoexplore), antipredator (Stationary) and other, less common behaviors (Table 1), using a freely available behavioral analysis program, JWatcher (vers. 1.0). We transformed the resulting data appropriately (log total duration and square root frequency), removed outliers, and ran correlation matrices on all possible combinations of dependent variables to meet the assumptions of both MANOVA (Tabachnick & Fidell 2001), and subsequent ANOVA (Martin & Bateson 2007), then analyzed using JMP (vers. 9; SAS Institute).

RESULTS

Frequency and total duration of behaviors were initially analyzed using MANOVA. The overall model in each case was highly significant (Frequency – Wilks' Lambda $F_{49,339.49} = 5.785$, $P < 0.0001$; Total Duration – Wilks' Lambda $F_{49,339.49} = 4.779$, $P < 0.0001$). There was a significant effect of both male age (Wilks' Lambda $F_{21,190.07} = 3.645$, $P < 0.0001$) and the presence of predator cues ($F_{7,66} = 30.611$, $P < 0.0001$) on the frequency of male behaviors, and the interaction was significant (Wilks' Lambda $F_{21,190.07} = 2.300$, $P = 0.0017$). Results were similar for the total duration data where there were significant effects of male age (Wilks' Lambda $F_{21,190.07} = 4.379$, $P < 0.0001$) and predator cues ($F_{7,66} = 37.398$, $P < 0.0001$) on the total duration of male behaviors, also with a significant interaction (Wilks' Lambda $F_{21,190.07} = 3.044$, $P < 0.0001$). The MANOVA analysis should be interpreted with caution as we found high negative correlation between the behavior "stationary" and other behavioral states. The accepted solution would be to remove the redundant variable (stationary) from analysis (Tabachnick & Fidell 2001), but since this behavior is also an important antipredator response, we felt strongly that it should be included. Further, the highly significant interaction terms make interpretation of the analysis difficult. For these reasons, we also analyzed each behavior independently using two-way ANOVA with Bonferroni adjustment (Tables 2, 3) (Tabachniek & Fidell 2001).

The presence of predator cues had a strong negative effect on frequency and total duration of active courtship behavior (Jerky Tap) of male *S. ocreata*, irrespective of male age (Tables 2, 3; Fig. 1). Frequency and total duration of Tap, a common

Table 2.—ANOVA results for mean frequency of behavioral bouts (number/300s trial) for male *Schizocosa ocreata*. (* Indicates significance after Bonferroni correction (α=0.007))

		Display Behaviors					
		Jerky Tap		Tap		Leg Raise	
Source	df	F	*p*	F	*p*	F	*p*
Male Age	3,72	0.082	0.970	7.634	<0.001*	10.413	<0.001*
Predator Cues	1,72	22.148	<0.001*	18.770	<0.001*	66.929	<0.001*
Age × Cues	3,72	0.875	0.458	4.201	0.009	6.117	<0.001*

		Other Behaviors							
		Chemoexplore		Grooming		Locomotion		Stationary	
Source	df	F	*p*	F	*p*	F	*p*	F	*p*
Male Age	3,72	1.278	0.289	5.324	0.002*	2.669	0.054	4.264	0.008
Predator Cues	1,72	5.080	0.027	0.000	1.000	17.092	<0.001*	8.599	0.005*
Age × Cues	3,72	0.847	0.473	0.383	0.766	2.734	0.050	2.993	0.036

Table 3.—ANOVA results for mean total duration (s) of behaviors for male *Schizocosa ocreata*. (* Indicates significance after Bonferroni correction (α=0.007))

		Display Behaviors					
		Jerky Tap		Tap		Leg Raise	
Source	df	F	*p*	F	*p*	F	*p*
Male Age	3,72	0.084	0.968	7.324	<0.001*	12.381	<0.001*
Predator Cues	1,72	22.401	<0.001*	5.045	0.028	83.359	<0.001*
Age × Cues	3,72	1.031	0.384	2.253	0.089	7.425	<0.001*

		Other Behaviors							
		Chemoexplore		Grooming		Locomotion		Stationary	
Source	df	F	*p*	F	*p*	F	*p*	F	*p*
Male Age	3,72	0.810	0.487	6.544	<0.001*	2.246	0.090	1.684	0.178
Predator Cues	1,72	0.894	0.348	0.000	1.000	8.362	<0.005*	10.513	0.002*
Age × Cues	3,72	1.094	0.357	1.007	0.395	1.711	0.172	1.315	0.276

male display trait correlated with active courtship, also declined significantly with male age, but was only slightly negatively impacted by the presence of predator cues (Tables 2, 3; Fig. 2). Leg Raises were significantly affected by both increasing male age and predator cues such that the behavior was performed almost exclusively in the presence of predator cues, but declined in both frequency and duration with increasing male age (Tables 2, 3; Fig. 3). The number and duration of bouts of Chemoexploratory behavior was largely unaffected by either predator cues or male age (Tables 2, 3), and while there was no detectable influence of predator cues on grooming, males groomed significantly more often and for longer periods as they aged (Tables 2, 3; Fig. 4). Neither locomotion nor time spent stationary was influenced by male age, but males spent more and longer periods stationary and fewer, shorter periods locomoting in the presence of predator cues (Tables 2, 3).

DISCUSSION

First, and importantly, the frequency and total duration of active, mate-seeking exploratory behavior (Chemoexplore) was consistent across trials, unaffected by advancing male age or the presence of predator cues (Tables 2, 3), so males were clearly able to detect the presence of conspecific female cues even under the influence of predator cues. All subsequent results, then, are unlikely to be a consequence of "masking" of conspecific female cues by predator cues. As suggested in previous studies of this species (Roberts & Uetz 2004b; Fowler-Finn & Hebets 2011), our results support that male *S. ocreata* are able to detect and respond to cues of potential predators by drastically modifying their behavior, even when no predator is physically present and predator cues are presented along with conflicting conspecific female cues. Further, increasing male age has a strong effect on some, but not all, male behaviors performed in response to female

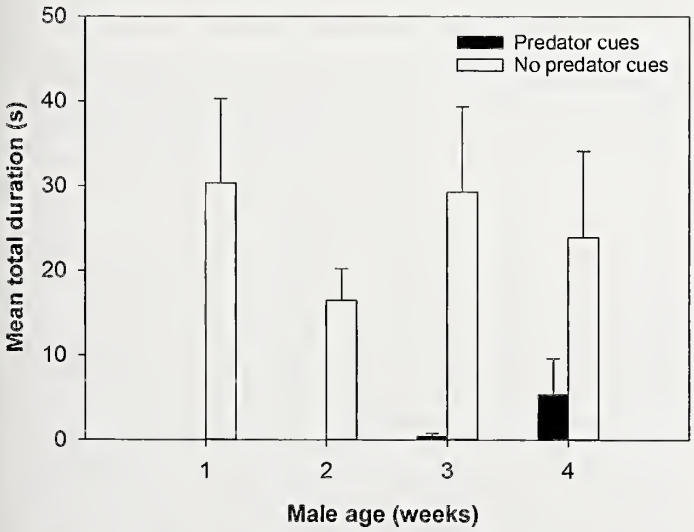

Figure 1.—Mean total duration (s) (+SE) of jerky tap behavior (active courtship) for male *Schizocosa ocreata* exposed to the silk and chemical cues of females in the presence or absence of predator cues.

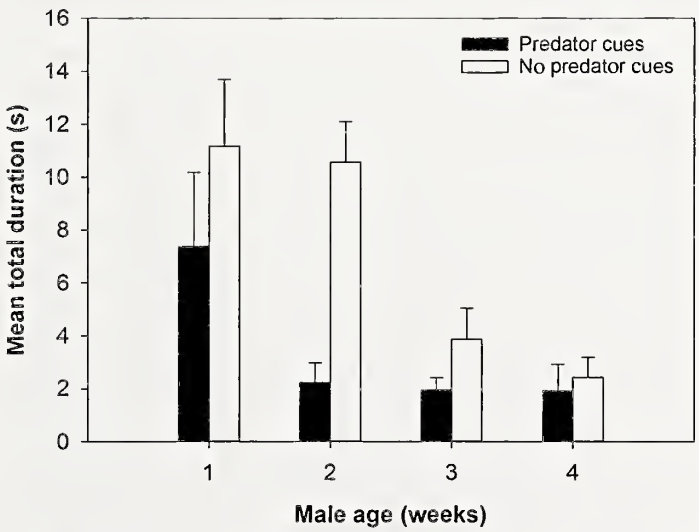

Figure 2.—Mean total duration (s) (+SE) of tapping behavior for male *Schizocosa ocreata* exposed to the silk and chemical cues of females in the presence or absence of predator cues.

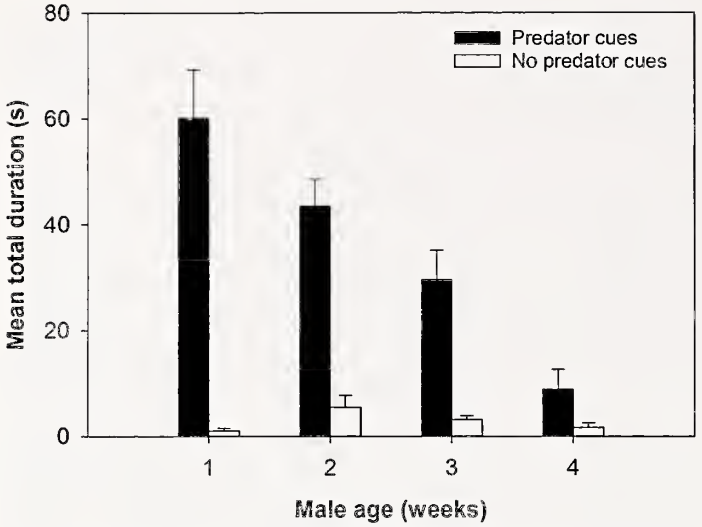

Figure 3.—Mean total duration (s) (+SE) of leg raise behavior for male *Schizocosa ocreata* exposed to the silk and chemical cues of females in the presence or absence of predator cues.

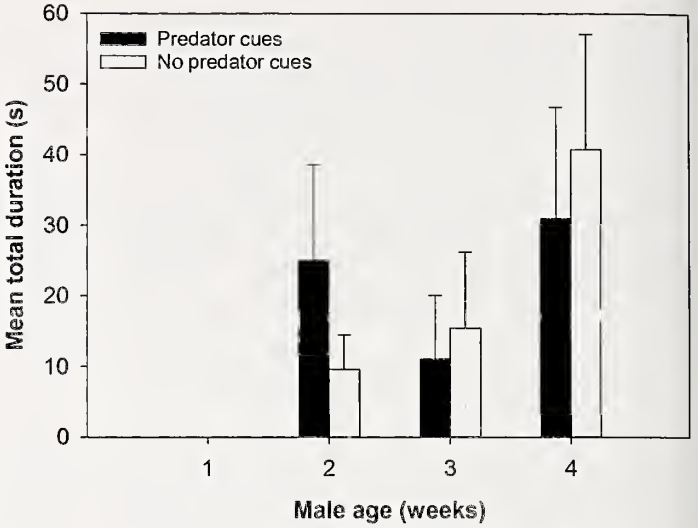

Figure 4.—Mean total duration (s) (+SE) of grooming behavior for male *Schizocosa ocreata* exposed to the silk and chemical cues of females in the presence or absence of predator cues.

cues. Counter to our terminal investment predictions, we found no meaningful interaction between increasing male age (senescence) and presence of predator cues, suggesting that male *S. ocreata* may not compensate for reduced reproductive potential by increasing use of risky, eomplex courtship behavior as they age.

Male *S. ocreata* exhibited equivalent levels of active courtship across all age categories when exposed to conspecific female cues alone (Fig. 1), suggesting that male courtship vigor may not measurably senesce with increasing age. Alternatively, and perhaps more likely, males may invest additional resources into active courtship to meet some threshold of vigor generally acceptable to receptive females (Delaney et al. 2007; Shamble et al. 2009; Byers et al. 2010), which is in line with predictions of terminal investment (Clutton-Brock 1984). In stark contrast to the effects of increasing age, males were unlikely to perform the prominent "Jerky Tap" courtship display behavior when cues of predatory *T. helluo* were present (Fig. 1). This does not support terminal investment under influence of predation, but does confirm similar findings of two previous studies. Roberts and Uetz (2004b), as part of an exploration of the species-specificity of female *S. ocreata* chemical cues, found that while males would occasionally court in response to silk and chemical cues of female spiders within, and even far outside, the wolf spider family (Lycosidae), they would not court in response to female *T. helluo* cues. Fowler-Finn and Hebets (2011), using number of body bounces as a proxy for male courtship, found that courtship was greatly reduced in the presence of *Tigrosa* spp. cues. Altogether, the results of these three studies suggest that a significant reduction in active courtship is an anti-predator response in this species. Complex, multimodal courtship by male *S. ocreata*, performed in this context-dependent manner, may benefit males in reproduction but must be severely costly in terms of increased predation risk (Roberts et al. 2007; Roberts & Uetz 2008).

While active courtship may be reduced or extinguished in the presence of predator cues across all age groups, younger males (one to two weeks post adult molt) instead adopted other, less "active" display traits (Figs. 2, 3). Leg Raise behaviors were performed almost exclusively in the presence of predator cues (Fig. 3), but were also clear indicators of male senescence with frequency and duration declining significantly with increasing age. Frequency and duration of tapping (Tap) also declined with age, and declined slightly faster in the presence of predator cues (Fig. 2). The most telling indicator of senescence in males is the significant inerease in grooming activity with age, whether or not predator cues were present (Fig. 4). Like many spiders, wolf spiders cease molting at maturity (Foelix 2011). Physical traits, such as the tufts of foreleg bristles male *S. ocreata* use in signaling to females, would be subject to wear as males age and thus an increase in maintenance behaviors like grooming is to be expected. Any shift in time allocation to grooming, though, must be balanced by shifts in other behaviors. If males maintain consistent courtship effort as they age, as it appears they do (Fig. 1), then this allocation shift may explain the decline in less critical display behaviors like leg raise or tapping (Figs. 2, 3).

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Spatial patterns and environmental determinants of community composition of web-building spiders in understory across edges between rubber plantations and forests

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Abstract. Rubber plantations in Southeast Asia have expanded greatly in recent decades, thereby increasing the amount of edges bounding natural forests. In this study, we focused on the effects of rubber plantation-forest edges on species diversity and abundance of web-building spiders. We also aimed to reveal environmental determinants that influence such patterns. We visually searched and collected spiders within 85 quadrats from October to January (heavy rain period), and 160 quadrats from May to September (light rain period). The quadrats were placed in five sites representing rubber plantations, rubber plantation-forest edge, and forest interior up to 150 m from the edge. We examined understory characteristics, microclimate, and potential prey within each quadrat. Certain species were abundant in rubber plantations, others were abundant at the edge or within the forest, and others showed no pattern. Species richness was not related to the edge whereas species diversity and total abundance of the spiders was higher in the rubber plantation and decreased at the rubber plantation-forest edge and into the forest interior. Temperature range and average temperature appear to drive the distribution patterns of species diversity and total abundance. Characteristics of understory, namely dry twigs and seedlings also tended to affect such patterns. Temperature probably affected the spiders' ability to maintain favorable body temperatures whereas dry twigs and seedlings probably provide reliable web support and suitable refuges.

Keywords: Alpha diversity, Araneae, edge effect, peninsular Thailand, temperature

Recent deforestation in Southeast Asia has been rapid due to the large-scale expansion of rubber plantations (Li et al. 2007). Replacing natural forests by rubber plantations can reduce biodiversity through habitat fragmentation as well as forest degradation (Zhai et al. 2012). An inerease of edge habitats and effects of edges are among the phenomena caused by the fragmentation; they may affect the distributions and the interactions of organisms in the ecosystem (Ries et al. 2004). Despite the situation mentioned above resulting in an increase of rubber plantations-forest edges, studies on the effect of this edge type on biodiversity are sparse.

Spiders are reliable bioindicators of environmental change in tropical ecosystems (Malumbres-Olarte et al. 2013). Web builders are sit-and-wait spiders that direetly use webs for capturing prey; they stay within the small range of their webs, so have small home ranges (Miyashita et al. 1998). They are highly sensitive to environmental changes (Lessard et al. 2010). Accordingly, the web builders appear to be suitable animal models to assess edge effeets, especially in small-scale ecosystems.

A number of studies have shown edge effects on arthropod abundance and richness (Bogyó et al. 2015; Lacasella & Zapparoli 2015). Their patterns along edge gradients varied depending on taxa or vegetation type (Albrecht et al. 2010; Rykken et al. 2011). Some studies reported no detectable edge effcts on the abundance of several groups of arthropods (Jabin et al. 2004). On spider diversity, most studies have shown positive edge effects (Gallé & Fehér 2006; Rodrigues et al. 2014) while a few studies have shown negative edge effects (Rodrigues et al. 2014) or no edge effects (Pearce et al. 2005). Also, a few studies have shown intermediate effects on spider diversity whereby at a plantation/pasture edge, spiders were more abundant relative to the forest plantation, but less abundant relative to the grass pasture (Downie et al. 1996). Specific studies on distribution patterns of web-building spider assemblages along edge gradients and environmental variables influencing these patterns are described by Baldissera et al. (2004, 2008). The edge between *Araucaria* forest and *Pinus* plantation did not significantly affect richness and abundance of web-building spiders (Baldissera et al. 2008), while the richness and abundance were positively affected by the edge between pasture and *Araucaria* forest. The latter pattern was positively influenced by vegetation species richness (Baldissera et al. 2004).

In this study we focused on the effects of rubber plantation-forest edge on web-building spiders. Our first objective was to investigate changes in species diversity, richness, and abundance of the spiders from rubber plantations across edges toward forest interiors. The edge effects can oceur both at community and species levels of spiders. At a community level, we expected that the spider diversity, richness, and total abundance would be highest in rubber plantations, where the understory habitat has relatively high complexity and density, and that it would decrease at the edge toward the forest which had a relatively sparse understory in our study area. At the species level, we expected that abundance of different species would vary in response to edge beeause of differenees in microhabitat preference. Although it is broadly known that environmental conditions influence spiders (Entling et al. 2007), the key environmental variables are not well understood, especially in the inter-habitat transition zones. Consequently, our second objective was to analyze environmental variables determining the patterns of spiders along the edge gradients. Based on previous research mentioned above, we

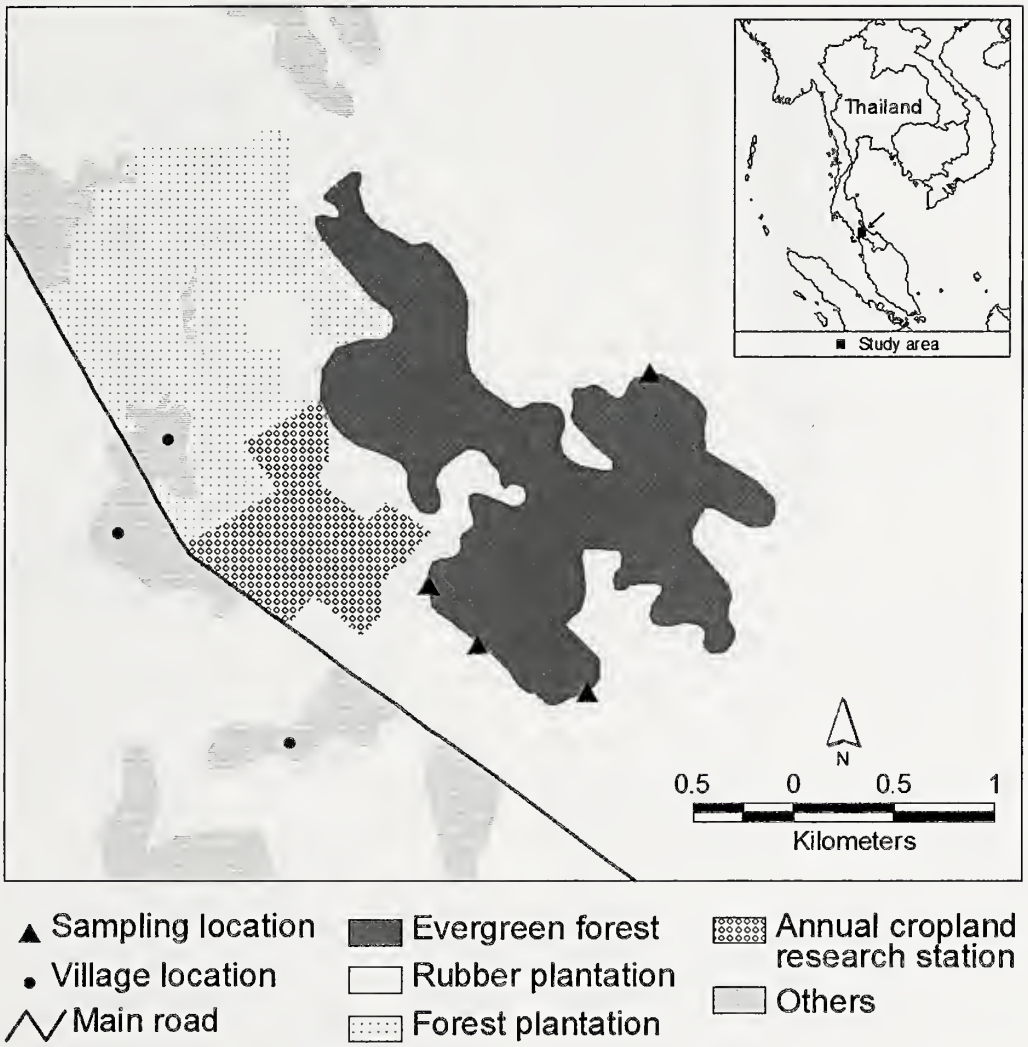

Figure 1.—A map of the study area, including the Khuan Khao Wang Forest Park (the dark grey component), and the rubber plantation zone (the white component). The black triangular symbols indicate the sampling locations where belt transects were set to extend from the rubber plantation into the forest.

predicted that vegetation complexity and density of understory would be the primary determinants.

METHODS

Study area.—The study was carried out in Khuan Khao Wang Forest Park (area = 3.26 km^2), Hat Yai District, Songkhla Province, southern Thailand (6°59″N, 100°18″E, 200 m a. s. l.) (Fig. 1). This forest park is a secondary forest remnant composed of semi-evergreen lowland trees, and has been naturally reforested for about 25 years since the termination of the logging concession. Logging began in 1970 and was terminated a few years later. Then, in 1995, the forest was assigned protected area status. The dominant trees in the forest park were *Barringtonia* spp., *Diospyros* spp., *Dipterocarpus alatus* Roxb. Ex G. Don, *Eugenia* spp., *Fagraea fragrans* Roxb., *Intsia* spp., *Lithocarpus* spp., *Morinda* spp., *Pterocarpus* spp., and *Shorea* spp. This proteeted area has a hill (200 m a. s. l.) and a few seasonal streams, and is surrounded by rubber plantations, forest plantations, a small area of cropland, a few palm plantations, and houses. Outside the boundaries of the forest park, the monoculture rubber plantations are dominant. The forest plantations have *Dipterocapus alatus* Roxb. ex G. Don, *Intsia palembanica* Miq., *Hopea odorata* Roxb., *Shorea roxburghii* G. Don, *Azadirachta excels* (Jack) Jacobs, and *Casuarina equisetifolia* J.R. & G. Forst. Most of the rubber plantations are mature (7–25 years old) with a canopy height of approximately 14 m; the rubber trees within each plantation are about the same age and height. Generally, the rubber trees are planted at 3 m intervals within each row, and 7 m spacing between the rows. The understory vegetation consists of grasses, sedges, herbs, ferns, vines, woody seedlings, and lianas, which are significantly denser in the rubber plantations than in the forests. Generally, the woody seedlings are dominant in the forest understory, while grasses are almost absent. In contrast, various species of grasses and herbs are the dominant vegetation in the rubber plantations. Human disturbances, including mowing and latex tapping, take place regularly in the rubber plantations. Traditionally, farmers slash or mow the understory in their plantations once a year, and they routinely walk the tracks along the rubber tree rows in order

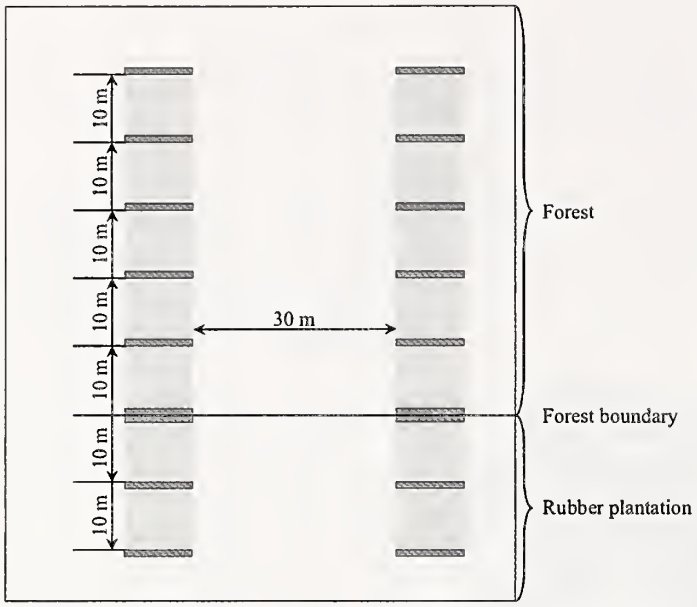

Figure 2.—The arrangement of 15 × 2 m sampling plots, crossing the forest boundary and extending to the forest interior and the rubber plantation. The understory, sapling, and tree densities were assessed by sampling these plots.

to tap the latex. The mean annual precipitation during 2003–2012 was 1890.3 ± 122.4 mm (mean ± SE). Generally, there are two seasons in the study area: dry and wet. Based on Mohr (1944), the dry season is from February to mid-April (mean monthly precipitation across 2003–2012 = 58.3 ± 15.2 mm). The wet season can be divided into two periods: the period of light rain from May to September (mean monthly precipitation across 2003–2012 = 86.1 ± 7.7 mm), and the period of heavy rain from October to January (mean monthly precipitation across 2003–2012 = 309.9 ± 34.6 mm) (Rattaphum meteorological station, unpublished data).

Edge determination.—The vegetation characteristics were assessed along paths from the rubber plantation into the forest, in order to determine the position and width of the edge zone between the rubber plantation and the forest. We selected rubber plantations of at least 15 years of age that had not had herbicides or insecticides applied for the last 10 years (based on interviews of rubber farmers), and had not had understory mowing during the last 6 months. We identified the line across which the vegetative contrast was strongest, approaching the forest from the rubber plantation (Cadenasso et al. 2003). We established belt transects of 15 m width, extending 20 m into the rubber plantation and 50 m into the forest from the forest boundary (the contrast line), spaced 30 m apart. We outlined 15 × 2 m plots in the rubber plantation and in the forest, on both sides of the forest boundary and then at every 10 m (Fig. 2), and counted saplings and trees (woody plants > 1.5 m tall) in the plots. We further outlined 1 × 1 m subplots at the center, as well as in the upper right and lower left corners of each plot, and assessed the understory in these subplots (see "Assessment of environmental variables" for details).

Study design.—We applied an interrupted belt transect sampling method on the rubber plantation across the edge toward the forest interior. Each transect was at least 50 m away from the outer bounds of the rubber plantation and the

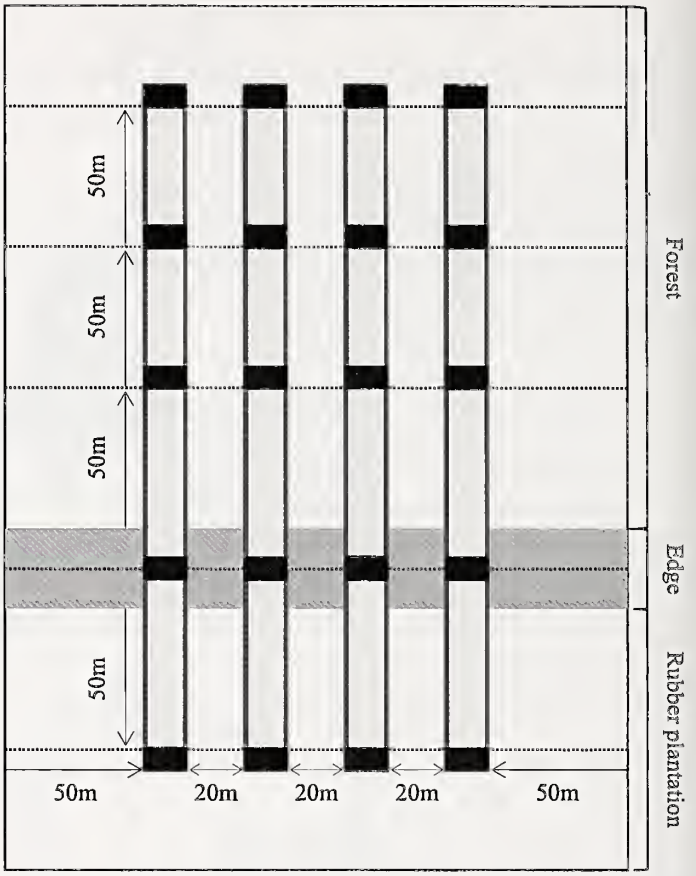

Figure 3.—The arrangement of 3 × 2 m quadrats for collecting spiders and assessing environmental variables at each site along belt transects spaced 20 m apart during the first session of data collection. A site was located at the edge, and others at 50 m from the edge into the rubber plantation, and 50, 100, and 150 m from the edge into the forest. During the second session of data collection, the same five sites along the transects were used, but this time 1 × 1 m quadrats were spaced 10 m apart along the transects.

forest (horizontal distance shown in Fig. 3). We conducted the study in two sessions. The first session, from October 2008 to January 2009 (in the period of heavy rain) was to examine whether the edges affect the distribution of spiders. We laid 17 belt transects (3 m wide) spaced 20 m apart, and placed 3 × 2 m quadrats to collect spiders at five sites along eaeh transect. The sampling sites on the transects were: at the edge, 50 m from the edge into the rubber plantation (RP); and 50 m (F050), 100 m (F100), and 150 m (F150) from the edge into the forest (Fig. 3). The second session, from June to September 2012 (during the light rain period) was to confirm an existence of edge effects and assess environmental determinants of spider distribution along the edge gradients. Because the heavy rains obstructed spider collection, we conducted data collecting of the second session in the light rains instead of the heavy rains. Spiders and data on environmental variables likely to affeet their distribution patterns (see "Spider sampling and identification" and "Assessment of environmental variables" for details) were collected. We laid 32 belt transects (1 m wide) spaced 10 m apart, and placed 1 × 1 m quadrats at every 50 m for five sites along each transect, in a similar arrangement as for the first session but at a different place to avoid

pseudoreplication (Fig. 3). We downsized the sampling quadrats in the second session, to be able to complete both spider and environmental variable samplings of each transect within the same day. In the rubber plantation, we placed sampling quadrats only between the rows of rubber trees and away from tracks, in order to avoid disturbance by walking farmers.

Spider sampling and identification.—Within eaeh quadrat, we found spiders during the daytime, on days without rain, from the ground up to 1.5 m height visually surveying all understories, saplings, trees, stones, and dry leaves/twigs/branches. We searched for spiders for 25 min. in the 3×2 m quadrats, and for 10 min. in the 1×1 m quadrats, to collect as many as possible. The time taken to eolleet spiders was excluded from the sampling time. Along each transect we randomized the order of quadrats for collecting spiders, on every collection day, to avoid temporal confounding effects related to the time of a day. Kleptoparasitic spiders were not included in this sampling. We identified mature spiders mainly on the basis of morphological characteristics, to the extent possible. For certain spiders, we used DNA analyses for identification, focusing on the mitochondrial cytochrome oxidase subunit I sampled from specimens preserved in 75% ethanol. All the procedures for DNA extraction, polymerase chain reaction, and sequencing, followed Tanikawa (2012), exeept for the DNA extraction kit. We used a FavorPrep Tissue Genomic DNA Extraction Mini Kit (Favorgen Biotech Corp, Ping-Tung, Taiwan). We applied the nomenclature after Platnick (2014). Specimens were stored in 75% ethanol in vials, and deposited in the Princess Maha Chakri Sirindhorn Natural History Museum at Prince of Songkla University, Hat Yai, Thailand.

Assessment of environmental variables.—We collected data on vegetation structure for edge determination. Although vegetation structure is well known to influence web-building spiders, microclimate (Sattler et al. 2010) and potential prey (Halaj et al. 2000) have been also suggested. Accordingly, to analyze environmental determinants of distribution patterns of the spiders along the edge gradients, we measured vegetation structure, microclimate and potential prey availability for evaluating determinants of spider distribution patterns (in the second session).

Vegetation structure: For edge determination, we counted the number of stems or trunks of trees and saplings in each plot. We quantified the density of understory vegetation by eounting leaves of grasses/sedges/ferns, all stems of vines and lianas, and primary stems of herbs/seedlings in each subplot.

For determinants of spider distribution patterns, we assessed densities of grasses, sedges, herbs, ferns, vines, lianas, seedlings, saplings, and trees by counting their buttresses, trunks, branches, stems, twigs, rachises, leaves, or inflorescences within eaeh quadrat. We then obtained a measure of vegetation complexity from the eombination of all plant categories above (McCoy & Bell 1991). We measured the cover percentage of eanopy by sighting with a cardboard tube with a crosshair through the eanopy. This was repeated at the center and in every corner of each quadrat (simple point intereept method: James & Shugart 1970). We estimated the cover percentage of litter on the ground, and measured the litter depth in all four corners and at the center of each quadrat. We counted the numbers of stones on the ground and also counted arboreal dead leaves/twigs/branches in the quadrats up to a height of 1.5 m.

Microclimate: We programmed data loggers, HOBO® U12 Temp/RH/Light/External Data Logger - U12 - 012 (Onset Corporation, Bourne, MA), to record temperature, relative humidity, and light intensity at 30 min intervals, and placed them in transparent plastic rain shelters at 1 m height in every site along eaeh transect, for a continuous period of 48 h. In each site, we randomly selected two from five points, four corners and at the center, within each quadrat. We also randomized the order of such two points for measuring microelimate in a quadrat and placed a data logger for 24 h at point one and moved to point two for continuing measurement another 24 h. From the resulting data, we ealculated the daily ranges (maximum − minimum) and average values for each of the microclimate variables (Vandergast & Gillespie 2004).

Prey availability: For insect sampling, we applied sticky traps made from 15×15 em transparent plastic pads coated with sticky glue. Within each quadrat along the transects, we placed the traps above the ground at 0, 0.5, 1.0, and 1.5 m heights, for 72 h. Insects captured by these traps were identified to order level following Borror et al. (1989). The trapped insects were counted, and their body lengths were measured. Dry biomass of each insect was estimated using the formula $W = 0.0305L^{2.62}$, where W is the dry mass in mg, and L is the length in mm (Lumsden & Bennett 2005).

Statistical analysis.—We applied the Shannon-Wiener diversity index to provide a measure of relative diversity of the spiders (Magurran & McGill 2011). To standardize species richness of spiders across sampling plots, we estimated rarefied species richness by using a function from the library "vegan" in R (Oksanen 2015). To designate dominant species, we calculated the proportion of individuals of each species divided by total number of individuals. We defined dominant species as those making up $\geq 3\%$ of individuals in the sample following Spiller & Schoener (1998). To compare the differences in spider diversity, species richness, and abundance of species in total and each dominant species between sites, we used one-way ANOVA, where "site" was used as a fixed factor. We checked the normality of spider data, using the Wilk-Shapiro test, and tested homogeneity of variance using Bartlett's test. The dependent variables were transformed by natural logarithms in cases where the data lacked normality or homogeneity of variance. We used Kruskal–Wallis tests when normality was not met. For post hoc multiple comparison tests, we applied Tukey's test following one-way ANOVA and Mann-Whitney U-test following Kruskal–Wallis tests. Because we used the Mann-Whitney U-test, which is a pairwise comparison for simultaneous inference, we adjusted the significance level by using the Dunn-Sidak procedure, in order to reduce the possibility of Type I errors (Quinn & Keough 2002). For dominant species, since their occurrences are not independent and we repeatedly applied the test on different species, we used Bonferroni correction to reduce the possibility of Type II errors (Cabin & Mitchell 2000). For spider diversity and total abundance that demonstrate patterns along edge gradients, we evaluated key environmental variables influencing the patterns. For abundance of the dominant species that

also demonstrated patterns along the edge gradients, we could not evaluate key environmental variables influencing their patterns because of too many zeros in the response variable data set.

We applied a Gaussian generalized linear model (GLM) with an identity link to evaluate the relationship between the environmental variables and spider diversity. For the spider abundance of all species combined, we applied zero-inflated models (Zuur et al. 2009), i.e., the ZIP or the ZINB models using the "psel" library in R (Jackman 2012). This approach was appropriate because our data had an excessive number of zeros. We used the MuMIn package in R (Barton 2012) to construct a set of alternative full models. We applied the Akaike Information Criterion (AIC) for model selection and presented only the best models (Burnham & Anderson 2002). To evaluate whether there are effects of spatial autocorrelation in parameters, we assessed spatial autocorrelation of the final model with correlograms using a spline function in the ncf package in R (Bjørnstad 2015). There was no significant spatial autocorrelation. We used each best model to predict the values of spider diversity and total abundance, as functions of the environmental variables, to assess the effect sizes of these variables (Martin et al. 2005). We computed the percentage of the effect size of each key environmental variable on spider diversity and the total abundance following Pilosof et al. (2012), dividing the predicted minimum value by the predicted maximum value of spider diversity and total abundance, and multiplying the result by 100. We performed all the analyses in R v.3.1.0 (R Core Team 2013).

RESULTS

Vegetation change and edge determination.—Understory density was higher in the rubber plantations than in the forests, decreasing sharply within 10 m of the forest boundary (from RP10 to F00, Fig. 4A). The tree density was lower in the rubber plantation than in the forest, and had a steep increase at the transition zone to the forest (from RP00 to F00) (Fig. 4B). Plant density changed conspicuously from 12 m within the rubber plantation to 2 m within the forest, measured from their boundary, and this defined an edge zone of approximately 14 m width (Figs. 2, 4).

Distribution of environmental variables.—The temperature range and seedling density differed significantly between the sites (Kruskal–Wallis tests, temperature range: $H_4 = 54.3$, $P < 0.001$; seedling density: $H_4 = 24.3$, $P < 0.001$, Fig. 5). The temperature range was wider at the RP than at the edge and in the forest. Likewise, seedling density in the RP was significantly greater than at the edge and in the forest. The average temperature and dry twig density did not differ significantly between the sites (Kruskal–Wallis tests, average temperature: $H_4 = 9.6$, $P = 0.05$; dry twig density: $H_4 = 8.7$, $P = 0.07$, Fig. 5).

Distribution pattern of web-building spiders.—During the first session (heavy rains), a total of 1753 spiders were collected including 917 (52.3%) juveniles and 836 (47.7%) adults. Adults belonged to 67 species of 14 families. Nine species were considered dominant, and these nine species accounted for 74% of total abundance. During the second session (light rains), a total of 908 spiders were collected, including 611 (67.3%) juveniles and 297 (32.7%) adults. Adults belonged to

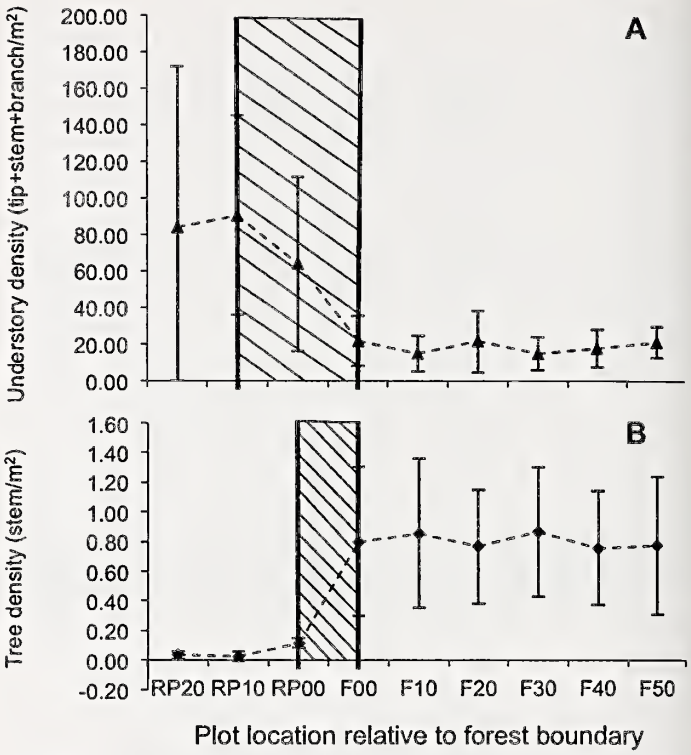

Figure 4.—Plots of the distribution of understory (A) and the tree density (B) along the transect extending from rubber plantation (RP) into forest (F). RP00, RP10, and RP20 are at distances 0, 10, and 20 m from the forest boundary to the rubber plantation, while F00 to F50 are at distances 0 to 50 m into the forest from the boundary. Points are means. Whiskers show SE.

50 species of 12 families. Nine species were considered dominant, accounting for 71% of total abundance.

During the first session (heavy rains), significant differences between rubber plantation and forest sites were found in *Crassignatha* sp2, Araneidae gen. sp3, and Mysmenidae gen. sp3 (Table 1). The abundance of *Crassignatha* sp2 was significantly higher in the F150 than in RP (Fig. 6A). The abundanee of Araneidae gen. sp3 was significantly higher at the edge than at RP and at F150 (Fig. 6B). The abundance of Mysmenidae gen. sp3 was significantly higher at RP than at the edge, F050, and F150 (Fig. 6C). The abundance of other dominant species, namely, Araneidae cf. *Nemoscolus* sp., *Leucauge argentina* (Hasselt, 1882), Linyphiidae gen. sp1, Mysmenidae gen. sp1, *Octonoba* sp1, *Zoma dibaiyin* Miller, Griswold & Yin, 2009, did not differ significantly between rubber plantation and forest sites.

During the second session (light rains), we found significant differences between the sites in Araneidae cf. *Nemoscolus* sp., Mysmenidae gen. sp1, and Theridiidae gen. sp1 (Table 1). The abundance of Araneidae cf. *Nemoscolus* sp. was significantly higher in the F150 than in RP (Fig. 6D). The abundance of Mysmenidae gen. sp1 was significantly higher at the edge than at F100 (Fig. 6E). The abundance of Theridiidae gen. sp1 was significantly higher at the rubber plantation than in the forest (Fig. 6F). The abundance of other dominant species, namely, Araneidae gen. sp3, *Belisana khaosok* Huber, 2005, Lycosidae gen. sp., Linyphiidae gen. sp1, Symphytognathidae gen. sp1,

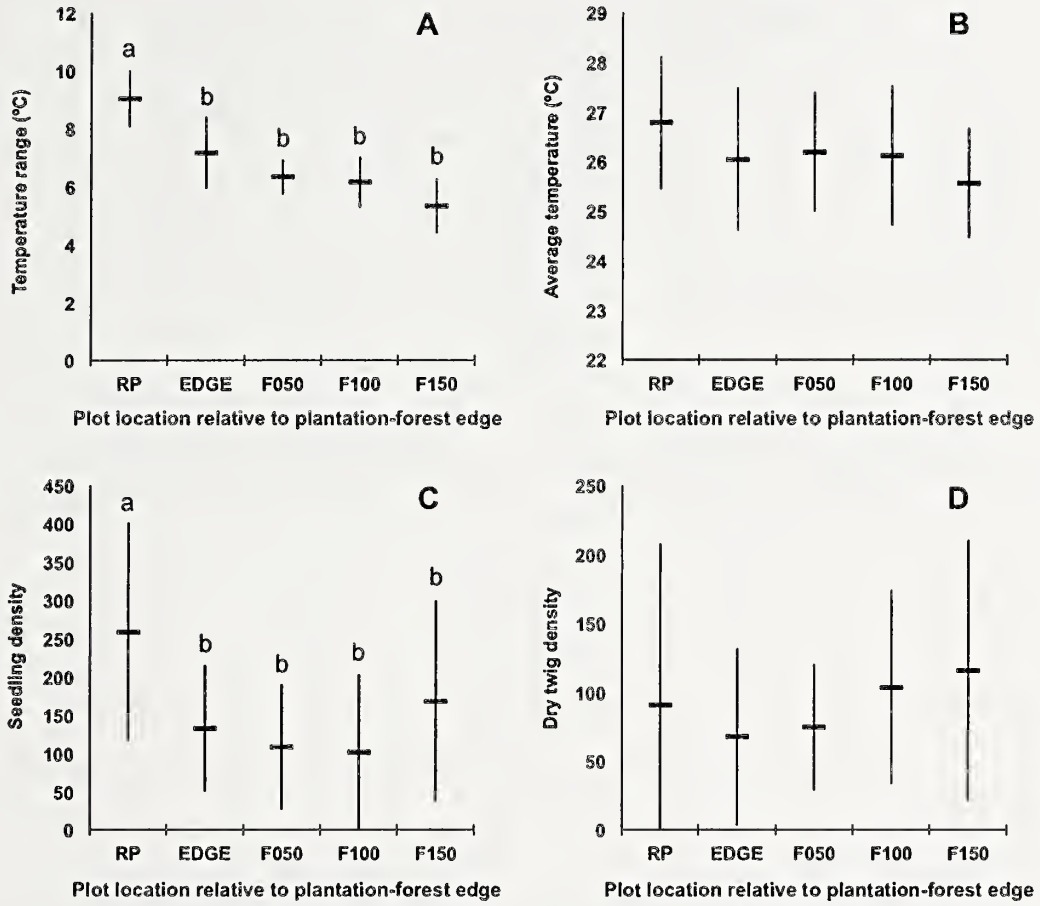

Figure 5.—The comparison of key environmental factors, temperature range (A), average temperature (B), seedling density (C), dry twig density (D), across the edge from rubber plantation into the forest. Bars are means. Whiskers are SE. Different letters indicate significant differences.

Table 1.—A list of dominant spider species, total abundance, and results of Kruskal–Wallis test (df = 4) comparing the abundance of each species between sites from rubber plantation and forest. Bold letters indicate significant differences between sites following a *post hoc* test.

Species	Total abundance	Kruskal-Wallis test H	*P*
Heavy rain period			
Araneidae cf. *Nemoscolus* sp.	67	5.4	0.25
Araneidae gen. sp3	33	19.9	< **0.001**
Crassignatha sp2	93	21.9	< **0.001**
Leucauge argentina (Hasselt, 1882)	38	11.9	< 0.05
Linyphiidae gen. sp1	74	2.4	0.66
Mysmenidae gen. sp1	229	3.6	0.46
Mysmenidae gen. sp3	21	31.2	< **0.001**
Octonoba sp1	23	6.2	0.19
Zoma dibaiyin Miller, Griswold & Yin, 2009	39	14.7	< 0.01
Light rain period			
Araneidae cf. *Nemoscolus* sp.	32	16.6	< **0.001**
Araneidae gen. sp3	9	0.8	0.94
Belisana khaosok Huber, 2005	10	6.1	0.19
Lycosidae gen. sp.	12	8.3	0.08
Linyphiidae gen. sp1	16	4.7	0.31
Mysmenidae gen. sp1	54	15.3	< **0.005**
Theridiidae gen. sp1	35	27.3	< **0.001**
Symphytognathidae gen. sp1	10	9.5	0.05
Symphytognathidae gen. sp2	34	9.5	0.05

Symphytognathidae gen. sp2, did not differ significantly between the sites (Table 1).

During the first session (heavy rains), the diversity of spiders differed significantly between the sites (Kruskal–Wallis test, $H_4 = 14.9$, $P < 0.01$). It was significantly higher in the RP than at F050 (Fig. 7A). Species richness and total abundance of spiders did not differ significantly between the sites (one-way ANOVA, richness: $F_{4,\ 95} = 1.0$, $P = 0.41$; abundance: $F_{4,\ 95} = 1.1$, $P = 0.36$, Fig. 7B, C). During the second session (light rains), the diversity and total abundance of spiders differed significantly between the sites (diversity: one-way ANOVA, $F_{4,\ 95} = 3.9$, $P < 0.01$; abundance: Kruskal–Wallis test, $H_4 = 16.4$, $P < 0.01$). The diversity of spiders was significantly higher at RP than at F100 (Fig. 7D). The abundance was significantly higher both at RP and at EDGE than at F100 (Fig. 7F). Species richness of spiders did not differ significantly between the sites (Kruskal–Wallis test, $H_4 = 4.0$, $P = 0.40$, Fig. 7E).

Variables influencing the distribution pattern of web-building spiders.—The average temperature and the temperature range were significant variables affecting total abundance and diversity of spiders (Table 2). Not only temperatures but also seedlings and dry twigs affected the total abundance and the diversity. The models suggest that a wider temperature range contributed to the increase in total abundance and diversity of spiders, while increasing the average temperature reduced the

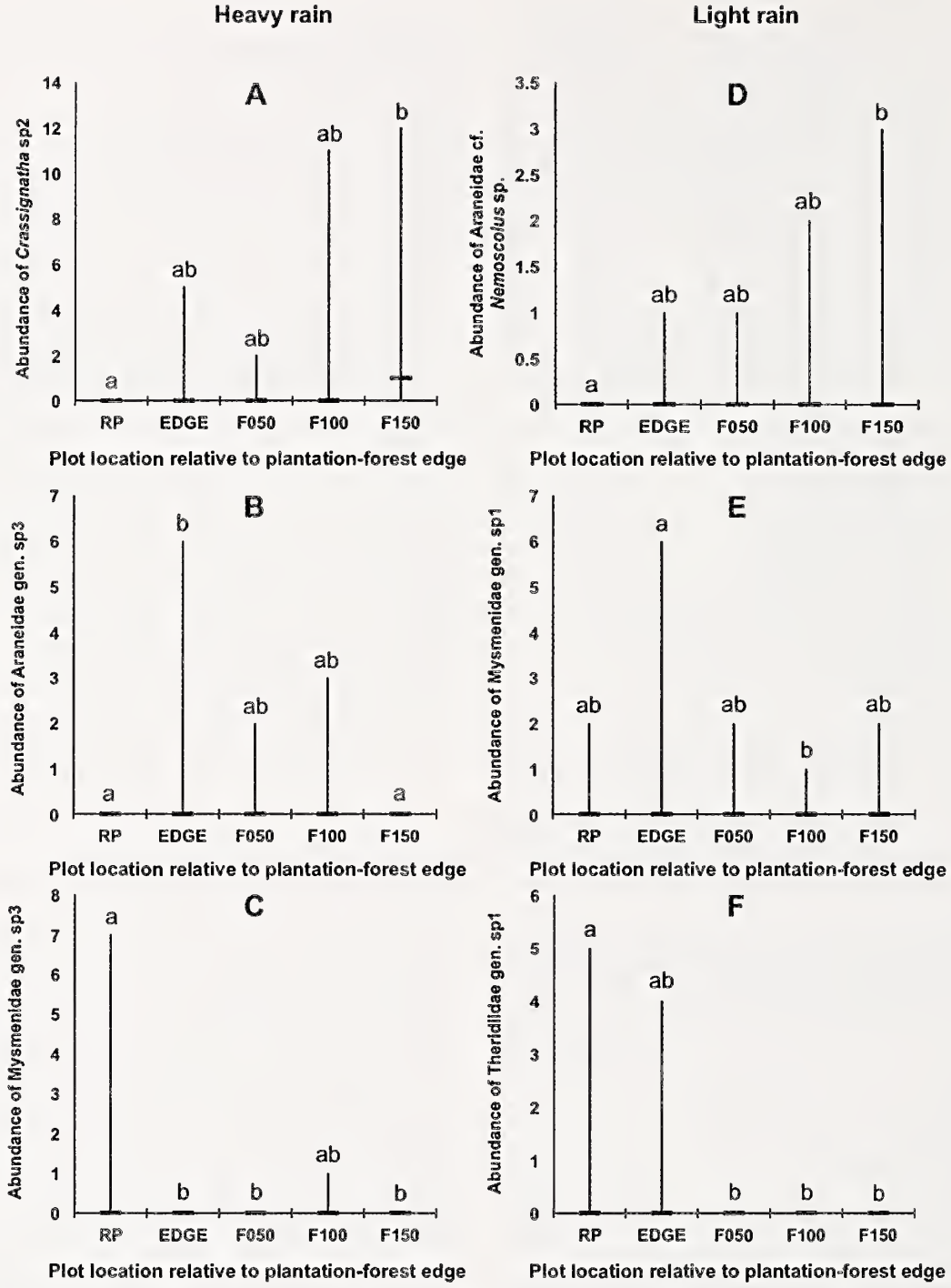

Figure 6.—Median values with range of abundance for the dominant species of spiders from rubber plantation into forest. Dashes are medians. Whiskers show ranges. Different letters indicate significant differences.

total abundance and the diversity. The numbers of seedlings and dry twigs positively influenced the total abundance and the diversity (Table 2). The zero-inflated negative-binomial model that we applied for total abundance of spiders did not show significant results (dry twig: $\beta = -0.274$, $Z = -0.963$, $P = 0.336$). The fitted GLM explained 19.3% of deviance in the diversity (Table 2). Species diversity was increased by 69% (from 1.15 to 1.67), 46% (from 1.24 to 2.71), and 47% (from 1.22 to 2.60) by temperature range, seedling abundance, and number of dry twigs, respectively. The diversity declined by 62% (from 1.68 to 1.05) with average temperature (Fig. 8). Temperature range, seedling abundance, and dry twigs increased the total abundance 22% (from 0.85 to 3.91), 9% (from 1.28 to 14.10), and 8% (from 1.15 to 14.15), respectively. The total abundance declined by 20% (from 3.47 to 0.68) with average temperature (Fig. 9).

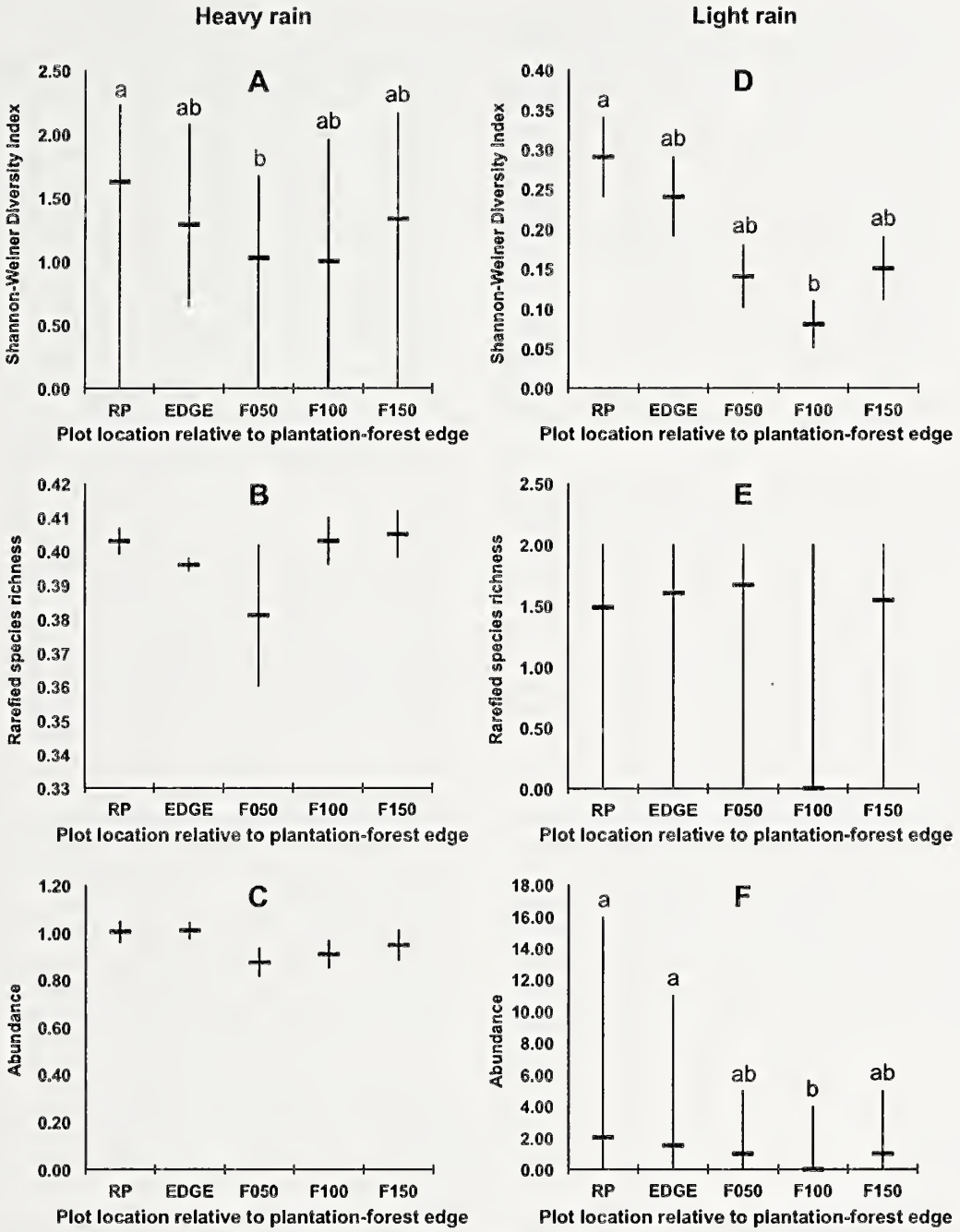

Figure 7.—The species diversity, the species richness, and the total abundance of web-building spiders from rubber plantation into forest. Mean values with SE are shown for the species diversity (D) during light rain period, and for the species richness (B) and the total abundance (C) during heavy rain period; dashes are means; whiskers are SE. Median values with range are shown for the species diversity (A) during the heavy rain period, and for the species richness (E) and the total abundance (F) during the light rain period; dashes are medians; whiskers indicate the range. Different letters indicate significant differences.

DISCUSSION

Effect of edge on the distribution pattern of web-building spiders.—Certain species of web-building spiders indicated the existence of the edge effect. As in previous studies (Baldissera et al. 2004; Vandergast & Gillespie 2004), the pattern of edge responses in abundance of spiders varied among different species. Edge influenced spider distribution in periods of both heavy and light rain, despite the fact that different taxa occurred in each period.

Crassignatha sp2, Mysmenidae gen. sp3, and Theridiidae gen. sp1, which were found only in a single period and responded to the edge, are probably sensitive to environmental change. The effects of edge on Araneidae gen. sp3, Araneidae cf. *Nemoscolus* sp., and Mysmenidae gen. sp1, which were found in both sampling periods varied. Araneidae gen. sp3 was influenced by edge in the period of heavy rain but not in the period of light rain. Araneidae cf. *Nemoscolus* sp. and Mysmenidae gen. sp1 were influenced by edge in the light rain

Table 2.—Summary of GLM testing the effect of four environmental variables on the diversity and total abundance of spiders. Bold values are significant at $P < 0.05$.

Type of model	Response variable Explanatory variables	Estimate	P
	Diversity		
Count model	Average temperature	−0.120	**<0.001**
	Temperature range	0.080	**<0.001**
	Seedlings	<0.001	**0.020**
	Dry twigs	<0.001	**0.040**
	Total abundance		
Count model	Average temperature	−0.393	**<0.001**
	Temperature range	0.253	**<0.001**
	Seedlings	0.001	**0.007**
	Dry twigs	0.002	**0.018**

period while neither species was influenced by edge in the heavy rain period. We postulated that these three species are intermediate in sensitivity to environmental change along the edge gradients. Linyphiidae gen. sp1 was found in both the periods of heavy and light rain and showed no edge effect, suggesting that it is insensitive to environmental change along the edge gradients.

This is the first report of an edge effect on the diversity of web builders. The distribution pattern of spider diversity showed an intermediate stage between the positive and negative effects of the edge between rubber plantation and forest. This was consistent across seasons, even though the magnitude of the effect varied seasonally. The positive effect of the edge between rubber plantation and forest was observed in spider abundance in the light rain period. This pattern is in aecord with the patterns in abundance of web-building spider assemblage described by Baldissera et al. (2004) and Vandergast & Gillespie (2004). No edge effects on spider abundance during heavy rains and species richness in both seasonal periods as revealed in the present study are similar to the results of Baldissera et al. (2008) but different from Baldissera et al. (2004).

Variables influencing the distribution pattern of web-building spiders.—The influence of the temperature range and the average temperature on the diversity and abundance of web-building spiders indicate that both these environmental variables mainly drive spider distribution patterns. Although Chaladze et al. (2014) and Kwon et al. (2014) showed influence of temperature on spiders, those studies did not examine small variations in temperature parameters between sites as in the present study. The response of web-building spiders to small variations in average temperature (26.2–27.3 °C) along the edge gradients observed here suggests the significant importance of temperature for determining spider distribution. In the present study, even though there is a small temperature range (5.7–8.8 °C) along the edge gradients, this is the most important variable positively driving distribution patterns of spider diversity and total abundance. In contrast to our results, Coyle (1981) reported that a wider range of temperatures decreased the diversity and the abundanee of web-builders in elear-felled temperate areas. In the present study, the maximum temperature range was 22.5–37.0 °C, and the daily range was typically 8.8 °C in the rubber plantations, which may not be too extreme for spiders. It is possible that the canopy of the rubber plantations alleviates the effeet of maximum temperature compared with the clear-felled areas. Based on our results, we postulate that web-building spiders prefer a wider range of temperature within the range of suitable temperatures.

A wider temperature range would support greater diversity and abundance. Different species may require different temperature optima for various essential activities, i.e., web building, prey capture, egg hatching, molting, development (Prestwich 1977; Li & Jackson 1996). Also, different species of spiders need appropriate ranges of ambient temperature to reach and maintain their favorable body temperatures (Krakauer 1972). The longer the activity, the more prey can

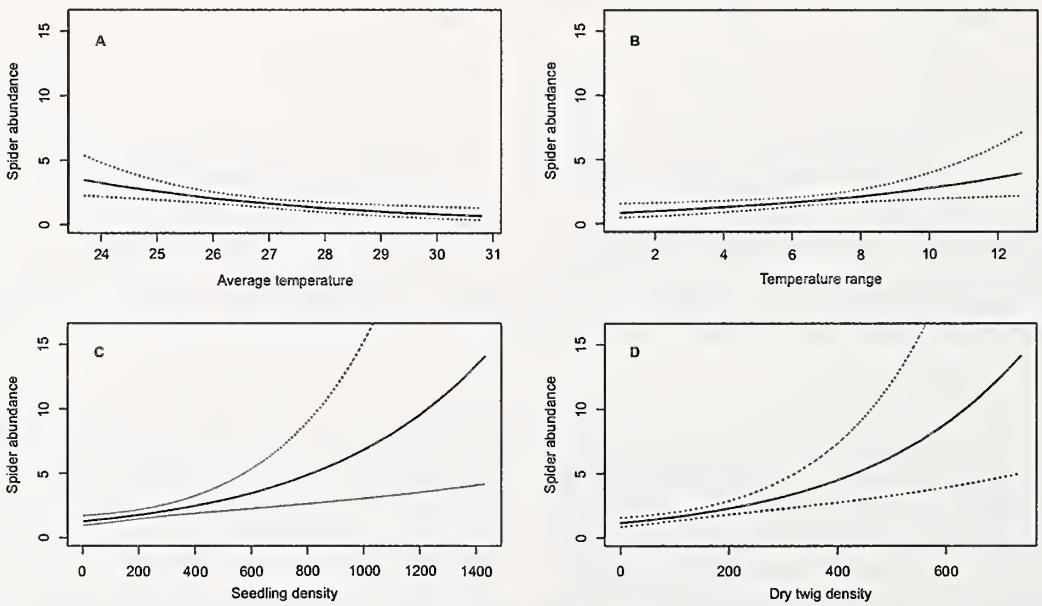

Figure 8.—Plots of the impacts of average temperature (A), temperature range (B), seedlings (C), and dry twigs (D), on the total abundance of web-building spiders of the top models. The solid lines are mean predicted values and the dashed lines indicate 95% confidence intervals.

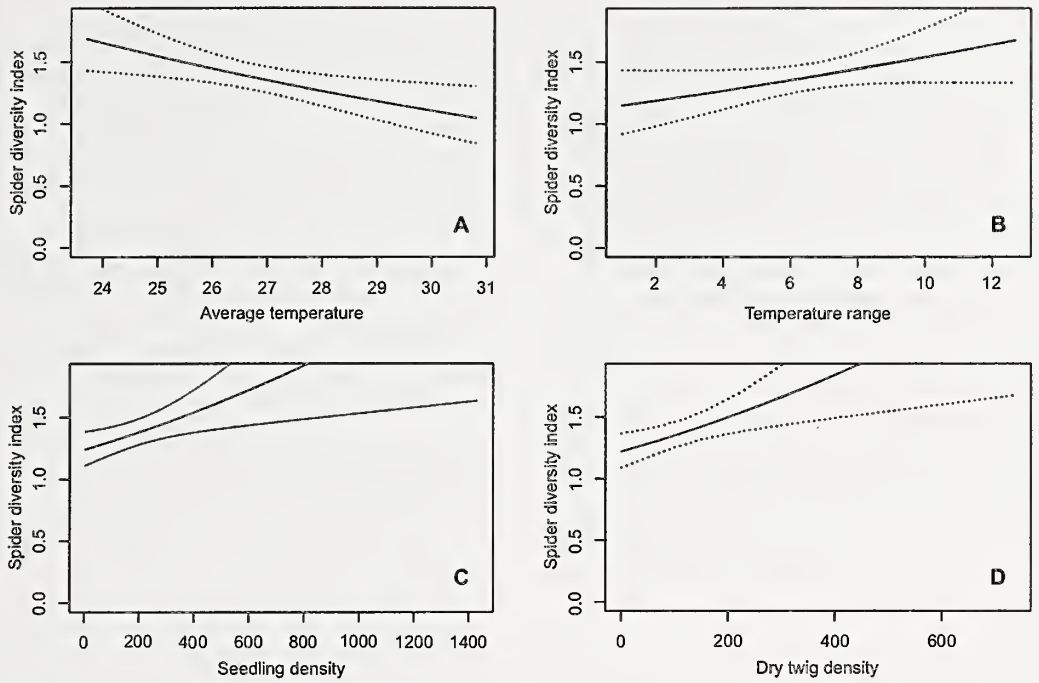

Figure 9.—Plots of the impacts of average temperature (A), temperature range (B), seedlings (C), and dry twigs (D), on the species diversity of web-building spiders of the top models. The solid lines are mean predicted values, and the dashed lines indicate 95% confidence intervals.

be captured and the more food is consumed; this contributes to early production of offspring and increased fecundity (Logan et al. 2006). Additionally, temperature affects silk properties (Yang et al. 2005). Certain spider species need specific temperatures to produce the best quality web, which is associated with the efficiency of prey capture (Barghusen et al. 1997). Previous research suggests that temperature is a signifieant factor driving distribution patterns of web-building spiders at a local scale (Finch et al. 2008), and our study suggests temperature may also be important on a very fine, microsite scale.

Only density of seedlings and dry twigs positively influenced the distribution patterns of spiders. The positive association with particular eharacteristics of the understory and spider diversity and total spider abundance is similar to Grill et al. (2005) and Blamires et al. (2007). Notably, the influence of twig density on the patterns also agrees with Gillespie (1987). The present study specifically indicates influence of seedling density on these patterns for the first time. Dry twigs and seedlings here could provide reliable architectural supports for various sizes and types of webs of most spiders (Miyashita et al. 2004) and proper refuges for spiders against their predators such as lizards (Hoffmaster 1982).

Edge effect penetration.—The edge effect between the rubber plantation and the forest was found up to 50 m into the forest during the heavy rain period, and up to 100 m during the light rain period. Thus, the penetration of the edge effect in the light rain period was deeper into the forest than in the heavy rain period. A key variable driving the patterns appeared to be the temperature; this could explain the edge effect being stronger during the dry season (Pohlman et al. 2009). Obvious changes in diversity and total abundance of web-building spiders across very short edge gradients in our study as compared with those found in beetle communities suggested that web-building spider assemblages could also be an efficient bioindicator of edge effects, especially on a small spatial scale (Ewers & Didham 2008).

Conservation implications.—Certain dominant species were abundant in the forest while others were abundant in the edge or rubber plantations. These patterns suggest that every position along the edge gradients is crucial for harboring particular species of web-building spiders. High diversity and total abundance of web-building spiders in rubber plantations compared to forest in the present study are the result of edge effects, and further study on such patterns in rubber plantation farther from forest is recommended. Since increased seedling and dry twig density in rubber plantations supports higher spider diversity, less disturbance to the understory could reduce losses of spider diversity (Beukuma et al. 2007). Generally, for rubber plantations, most farmers frequently clear the understory with herbicides or meehanical cutting. However, a few farmers, who practice agroforest rubber plantation, leave the understory undisturbed and plant more forest tree seedlings. It would be particularly informative to assess spider diversity between these different practices of rubber plantations.

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First record of a representative of Ballarrinae (Opiliones: Neopilionidae), *Americovibone remota* sp. nov., from New Zealand

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Abstract. *Americovibone remota* sp. nov. is described as the first New Zealand representative of the Ballarrinae, a Gondwanan-distributed group of harvestmen (Arachnida: Opiliones: Palpatores), from a female collected at Dart Hut in Mount Aspiring National Park. Though closely allied by external and ovipositor morphology to *Americovibone lanfrancoae* Hunt & Cokendolpher, 1991 of southern South America, *A. remota* lacks the reflexed pedipalpal tibia previously regarded as characteristic of the Ballarrinae. The genus *Americovibone* is restricted to austral *Nothofagus* forests which have a similar trans-Pacific distribution.

Keywords: Arachnida, Palpatores, taxonomy, biogeography, Gondwana

http://zoobank.org/References/EEE95A2C-9951-4EF3-B7D7-82EA70A09671

The Ballarrinae are a highly distinctive but little-studied group of long-legged harvestmen (Opiliones: Palpatores) found in continents of the Southern Hemisphere. They are immediately recognizable from their distinctive pedipalps, which have an elongate patella, reduced tibia and lack a tarsal claw (Hunt & Cokendolpher 1991). They are also noteworthy for including some of the smallest of all Opiliones, with body lengths less than 1.5 mm in some speeies (Hunt & Cokendolpher 1991).

When first described by Hunt & Cokendolpher (1991), the Ballarrinae exhibited a near-classic Gondwanan distribution, with species found in southern parts of eaeh of South America, Africa and Australia. However, they have hitherto appeared to be curiously absent from New Zealand. Other Gondwanan-distributed groups of Opiliones, such as Pettalidae (Boyer & Giribet 2009), Triaenonychidae (Forster 1954) and Enantiobuninae (Taylor 2011; Fernández et al. 2014), are diverse in the region and make up the greater part of the local Opiliones fauna. Recently, while sorting through material on loan from the New Zealand Arthropod Collection (NZAC), I discovered a specimen of Ballarrinae in a collection from the Dart Hut in New Zealand's Mount Aspiring National Park. Though only a single female specimen was available, this represents a significant development in our understanding of the New Zealand harvestman fauna.

The New Zealand specimen is very similar to the southern Chilean species *Americovibone lanfrancoae* Hunt & Cokendolpher, 1991 and is here described as a second species of the same genus. This implies a closer relationship of the New Zealand Ballarrinae to South America than to the Australian genera *Plesioballarra* Hunt & Cokendolpher, 1991, *Arrallaba* Hunt & Cokendolpher, 1991 and *Ballarra* Hunt & Cokendolpher, 1991.

The specimen was sourced from the New Zealand Arthropod Collection (NZAC), Landcare Research, Auckland. It was examined using a Nikon SMZ1500 stereo microscope, and photographs and measurements were taken using the NIS-Elements D 4.00.03 program. The ovipositor was removed and partially cleared using 50% lactic acid, and the ovipositor, a separated chelicera, and the original specimen were examined using a Leica DM2500 compound microscope. The ovipositor and separated chelicera were retained in a microvial with the original specimen. Coloration is described as preserved in alcohol. Measurements are reported in millimeters (mm).

Family Neopilionidae Lawrence, 1931
Subfamily Ballarrinae Hunt & Cokendolpher, 1991
Genus *Americovibone* Hunt & Cokendolpher, 1991

Americovibone Hunt & Cokendolpher, 1991: 165.

Type species.—*Americovibone lanfrancoae* Hunt & Cokendolpher, 1991, by original designation.

Remarks.—*Americovibone remota* sp. nov. is consistent with the description of *Americovibone* provided by Hunt & Cokendolpher (1991). Important diagnostic features of this genus include: chelicera with ventral spur at base of segment I; pedipalp with patella longer than tibia and tarsus, femur and patella of female pedipalp pseudosegmented; leg claws with lateral teeth; ovipositor corpus with more than two segments, two spermathecae present. *Americovibone remota* also has a spermathecal morphology very similar to those of *A. lanfrancoae*.

Americovibone remota sp. nov.
(Fig. 1)
http://zoobank.org/NomenclaturalActs/F6D622B7-A474-4553-BE09-683D85C12E2D

Type material.—*Holotype female*. NEW ZEALAND: Dart Hut, Mount Aspiring National Park, 44°32′S 168°33′E, 920 m, 13–14 February 1980, J. S. Dugdale, pan trap in bush (NZAC).

Etymology.—From the Latin *remotus*, remote, in reference to both the remoteness of the type locality in New Zealand, and the species' remoteness from its closest ally in South America.

Diagnosis.—*Americovibone remota* differs from *A. lanfrancoae* in having the pedipalpal tibia not reflexed on the patella,

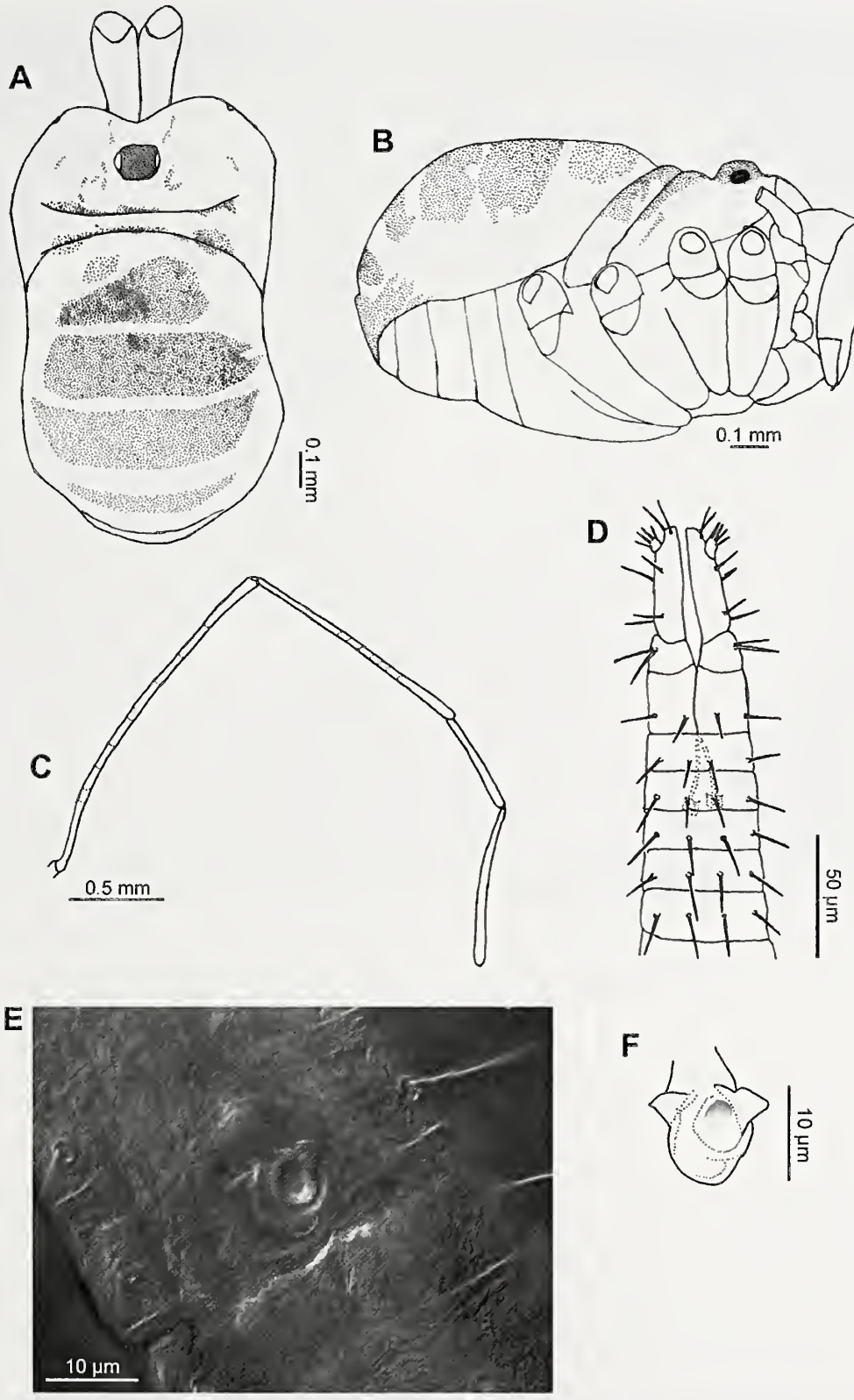

Figure 1.—*Americovibone remota* sp. nov., holotype female. A. Body, dorsal view; B. Body, lateral view; C. Right pedipalp, lateral view, setae omitted; D. Ovipositor, showing position of spermathecae as dotted lines; E. Photograph of spermatheca; F. Line diagram of spermatheca.

with the dorsal angle between the two segments greater than 180°. As in other Phalangioidea, the patella-tibia junction of the pedipalp in Ballarrinae is able to flex laterally but not dorsoventrally (Wolff et al., in press), so the difference in palpal disposition between the two species is not an artefact of preservation. The two species may also differ in color pattern, with *A. lanfrancoae* illustrated by Hunt & Cokendolpher (1991) as having the propodeum medially brown, and the

dorsum of its opisthosoma more extensively pale with the brown transverse striping broken medially. However, this should be treated with caution due to the reported poor condition of the *A. lanfrancoae* type specimens. Comparison of body size between *A. remota* and *A. lanfrancoae* is also impeded by the condition of the single known female specimen of the latter, in whieh the main body is distorted and the legs missing (Hunt & Cokendolpher 1991). However, differences between cheliceral and pedipalpal measurements of the two species are minimal except that the pedipalpal femur and tarsus are relatively longer in *A. lanfrancoae* (1.97 and 0.98 mm, respectively; patella 1.4× length of tarsus in *A. lanfrancoae* vs 1.6× in *A. remota*).

Description (female holotype).—Prosoma length 0.29, prosoma width 0.73, body length 1.18. Dorsum unarmed, without prominent setae. Ozopores small, round, not raised on lobes. Propodeum cream-colored; ocularium black. Mesopeltidium, metapeltidium and dorsum of opisthosoma mostly mottled brown, with cream-colored transverse stripes and cream-colored lateral margins on opisthosoma. Venter cream-colored, without prominent setae; coxapophysis II angled slightly rearwards. *Chelicerae*: Segment I 0.25, segment II 0.46. Unarmed. Base of segment I with ventral spur; spinose scales absent. Fingers relatively slender, bent mesad in frontal view; teeth all small. *Pedipalps* (Fig. 1C): Femur 1.89, patella 1.34, tibia 0.59, tarsus 0.84. Femur more than 1.5 times main body length, with ten pseudosegments. Patella more than two times length of tibia, with eight pseudosegments whose boundaries are concentrated towards midlength; patella with small swelling retrodistally. Tibia not reflexed relative to patella; dorsal angle between patella and tibia slightly more than 180°. Tarsus evenly concave on dorsal margin. Sparse plumose setae along entire length of pedipalp, becoming denser distally on tarsus; plumes restricted to one side of each seta, reaching about halfway down length of each seta on femur to tibia, extending further down each seta on tarsus. Few non-plumose setae present at apex of tarsus. Tarsal claw and microtrichia absent. *Legs*: Leg I femur 1.57, patella 0.33, tibia 1.38; leg II femur 2.96, patella 0.34, tibia 2.92; leg III femur 1.58, patella 0.31, tibia 1.41; leg IV femur 2.40, patella 0.36, tibia 2.32. All segments unarmed. Femora of all legs with, respectively, 6, 12, 4–5 and 7–8 pseudosegments; tibia II with 12 pseudosegments, tibia IV with 3 pseudosegments, tibiae I and III not pseudosegmented. Metatarsi elongate but not pseudosegmented. Tarsal claws of legs I, III and IV with sharp bend near base; dentate lateral carina present on either side; tarsal elaw of leg II weaker, without lateral carina. *Ovipositor* (Fig. 1D–F): Furca three-segmented, distal segment of furca elongate (about four times as long as wide). Two spermathecae present in second and third segments; spermathecae consisting of short loop with lateral extension on each side.

Remarks.—As the new species described herein is more similar to *A. lanfrancoae* than any other species of Ballarrinae, it is provisionally assigned to the same genus pending the discovery of male specimens. It might be questioned whether it is appropriate to describe a new species of Opiliones from a single female specimen, considering the pre-eminent position of male genitalic characters in Opiliones taxonomy (see e.g., Macías-Ordóñez et al. 2010; Pérez-González 2011; Pinto-da-Rocha et al. 2012), the presence in many groups of Opiliones of significant sexual dimorphism, and the potential difficulty of distinguishing females of closely related species (see e.g., Taylor 2004). However, this particular example represents the first record in New Zealand of a significant group of Opiliones whose presence there has not hitherto been recognized. Many parts of the south-western South Island of New Zealand are of limited accessibility, and so have not been extensively collected. The type locality of *A. remota*, Dart Hut, is positioned in the eastern part of Mount Aspiring National Park at the junction of Snowy Creek and the Dart River (Fig. 2). Dart Hut is two days' walk from the nearest road, with the entire Rees-Dart track taking four or five days (Department of Conservation 2013).

Significant sexual dimorphism has not yet been recorded from Ballarrinae, though males are generally smaller than females (Hunt & Cokendolpher 1991). The main external differences between *A. lanfrancoae* males and females are that male pedipalps lack the pseudosegments found in females, and the male pedipalpal tarsus is dorsally convex rather than coneave. Hunt & Cokendolpher (1991) did not identify any significant differences between the sexes in coloration, ornamentation or cheliceral development.

As noted above, the close similarity of *A. remota* to *A. lanfrancoae* suggests a closer relationship of New Zealand Ballarrinae to South American than to Australian species. Though less common than the converse, other examples of this pattern of biogeographic relationships inelude the plant genus *Aristotelia* (Elaeocarpaceae) and certain members of the midge subfamily Podonominae (Diptera: Chironomidae) (Crisci et al. 1991). However, it is also noteworthy that Dart Hut is positioned in a beech (*Nothofagus*) forest (map in Sommerville et al. 1982), which is also the known habitat for the South American species (Hunt & Cokendolpher 1991). *Nothofagus* has only a relictual distribution in mainland Australia, being found in Tasmania, southern Victoria and across the New South Wales-Queensland border (Knapp et al. 2005). Where habitat has been recorded, Australian Ballarrinae are mostly known from *Eucalyptus* woodlands, though *Ballarra cantrelli* Hunt & Cokendolpher, 1991 and *Plesioballarra crinis* Hunt & Cokendolpher, 1991 are possibly within the range of *Nothofagus moorei* (Hunt & Cokendolpher 1991). No Ballarrinae have as yet been described from Tasmania.

It is also possible that loeal extinctions have complicated the biogeography of Ballarrinae. The endemic New Zealand bat genus *Mystacina* is more closely related to South American taxa in the Noctilionoidea than to any living Australian bats (Teeling et al. 2005). Nevertheless, the fossil record supports an Australian origin for *Mystacina*, with its probable sister genus *Icarops* being present in the Miocene of northern Australia (Hand et al. 1998).

Though the Ballarrinae were initially united on the basis of their distinctive pedipalpal morphology (Hunt & Cokendolpher 1991), a recent molecular analysis of Palpatores has questioned the monophyly of ballarrines (Groh & Giribet 2015). In this study, the South African *Vibone vetusta* Kauri, 1961 failed to form a clade with the Australian *Ballarra longipalpus* Hunt & Cokendolpher, 1991. Conversely, *B. longipalpus* and the South American *A. lanfrancoae* did form

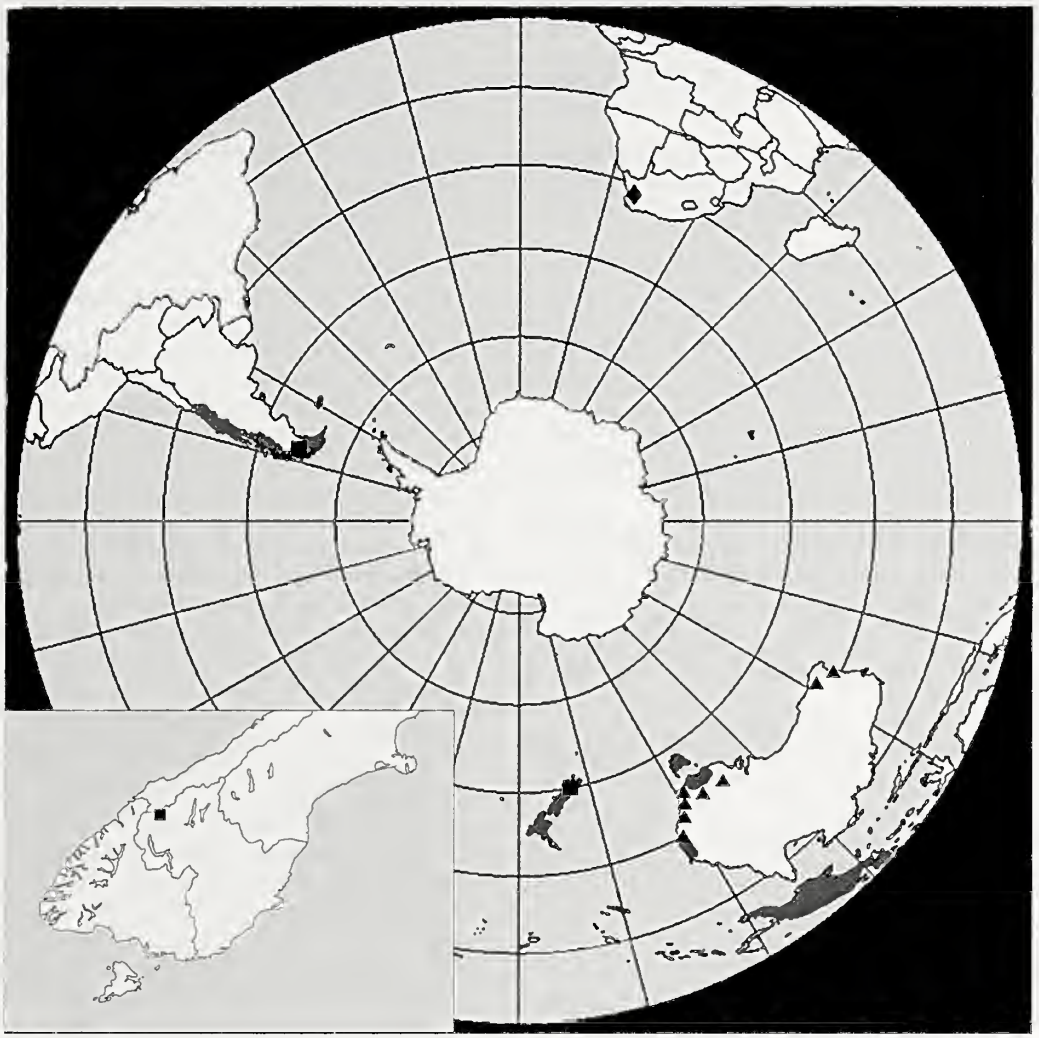

Figure 2.—Map of Southern Hemisphere, showing distribution of Ballarrinae and *Nothofagus*. Symbols indicating known localities of main Ballarrinae subgroups (Hunt & Cokendolpher 1991): square = *Americovibone*; diamond = *Vibone*; triangle = Australian clade (*Ballarra*, *Arrallaba* and *Plesioballarra*). Distribution of *Nothofagus* shown in grey (based on Knapp *et al.* 2005, modified for scale). Inset: southern South Island, New Zealand, with black square indicating type locality of *Americovibone remota*.

a clade when included in a morphological analysis of Neopilionidae by Taylor (2013). However, neither of these analyses included more than two species of Ballarrinae nor had the Ballarrinae as their main focus. The Australian Ballarrinae are united by their distinctive male genital morphology, with a barbed process on the left ventral side of the penis (Hunt & Cokendolpher 1991). This process is absent in *A. lanfrancoae*. The male of *V. vetusta* is unfortunately unknown but it is noteworthy that the ovipositor morphology of this species, as described by Kauri (1961), is unique in the Phalangioidea, with the main body of the ovipositor largely unsegmented.

The absence of a dorsal reflexion between the patella and tibia of the pedipalp in *A. remota*, previously regarded as a defining feature of the Ballarrinae, gives credence to the possibility that the unusual ballarrine palpal morphology may have evolved independently. Glandular setae on harvestmen pedipalps produce sticky secretions that are used in prey capture (Wolff et al. 2014), and it is possible that the 'ballarrine' pedipalp represents a convergent adaption to the aetive predation of fast-moving prey such as springtails. Further investigation into the monophyly of this group is required.

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Mechanical properties of male genitalia in *Leiobunum* harvestmen (Opiliones: Sclerosomatidae)

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Abstract. The morphology of arthropod intromittent organs evolves rapidly and is often species specific, phenomena widely attributed to sexual selection. Similar patterns in biomechanical properties may also exist, but practical challenges in manipulating small structures and measuring minute forces has impeded experimental biomechanical analysis. Here we describe a device that displaces a small structure while measuring its resistance, and use it to examine the biomechanics of penile flexure in the eastern North American harvestman genus *Leiobunum* C.L. Koch, 1839. Several *Leiobunum* lineages have lost primitive penis-associated nuptial-gift sacs and have gained apparent female pregenital barriers, a co-evolutionary pattern consistent with shifts from precopulatory enticement to more-antagonistic strategies. We tested for an association between losses of nuptial-gift sacs and increases in penile flexural resistance using five sacculate and five non-sacculate species. We measured three mechanical variables—resistance force, elastic efficiency and viscoelastic relaxation time—under lateral, dorsal, and ventral flexion. Our functional assumptions about sacculate and non-sacculate penes anticipated two biomechanically-defined species clusters, but three were found: a diverse sacculate group, a monophyletic non-sacculate group and an unanticipated mixed group. This work demonstrates that experimental genital biomechanics in arthropods is possible, and we discuss the functional implications of our results.

Keywords: Reproduction, viscoelasticity, elastic efficiency, phylogenetic comparative methods

Explaining the remarkable diversity of reproductive structures in arthropods and other animals is a perennial goal of evolutionary biologists (Day & Young 2004; Leonard & Cordoba-Aguilar 2010). Attention has centered on the often species-specific and sometimes exaggerated or complex traits of males (Hosken & Stockley 2004), although several recent authors have highlighted the importance of genital variation in females as well (Brennan et al. 2007; Sánchez et al. 2011; Tanabe & Sota 2013; Ah-King et al. 2014). The persistent bias toward research on male structures, especially intromittent organs, likely reflects their proven value for delimiting species (Edwards & Knowles 2014), their relatively rapid rate of evolution (Cayetano et al. 2011; Cassidy et al. 2014; Masly & Kamimura 2014), and the numerous evolutionary factors that have been invoked to explain their diversity (Leonard & Cordoba-Aguilar 2010), including female preference (Kokko et al. 2003), sperm competition (Parker et al. 2013), and cryptic female choice (Eberhard 1996; Albo et al. 2013). An understanding of the contributions that different selection mechanisms have made in shaping genitalic diversity should benefit from detailed information about the mechanical properties of genitalia (Cayetano et al. 2011), but existing information is largely based on inferences drawn from statie anatomy or associated behavior rather than from experimental measurement of biomechanical variables (Bonduriansky & Day 2003; Márquez & Knowles 2007). Consequently, despite active interest in the roles of female enticement and coercion as male mating strategies, there is little information about the intrinsic ability of male intromittent organs to respond mechanieally to female movement or to overcome female resistance during antagonistic interactions (but see Brennan et al. 2010). Here we focus on the mechanical properties of penes in the eastern North American species of harvestmen from the genus *Leiobunum* C.L. Koch, 1839 (Fig. 1), a clade for which reproductive diversity is increasingly well documented (Burns et al. 2012, 2013; Fowler-Finn et al. 2014; Burns & Shultz 2015).

Harvestmen are unusual among arachnids in having a true penis and in mating face to face (Machado et al. 2015; Fig. 1). The reproductive structures in both sexes are enclosed within a pregenital chamber that occupies the ventral part of the abdomen and opens anteriorly just posterior to the mouth. This chamber is enclosed ventrally by a large sclerite, the genital operculum, which articulates with the abdomen posteriorly via a transverse hinge to open and close like a trapdoor. The penis is essentially a cuticular tube that usually has a subterminal joint that divides it into a long proximal shaft and short distal glans, which has a thin terminal stylus that bears the small primary genital opening. The glans-shaft joint is operated by a bi- or multi-pinnate muscle that arises from the walls of the shaft and inserts via a long tendon on the ventral surface of the joint. The penis is externalized anteriorly by the combined effects of protractor muscles and hydraulic eversion of the flexible walls of the pregenital chamber.

Two basie types of penes have been distinguished based on the presence or absence of a subterminal pair of cuticular sacs (Fig. 1A). The sacs carry a male-generated nuptial gift that may be accessed orally by the female early in mating (Fig. 1D), a behavior that was often confused with copulation by early naturalists due to the proximity of the mouth and pregenital opening. Penile sacs were lost several times in *Leiobunum* (Burns et al. 2013); losses typically accompanied by the evolution of a sclerotized pregenital barrier in females. These correlated transformations suggest that female enticement via nuptial gifts was important in the primitive premating strategy in *Leiobunum* but was replaced multiple times by other mechanisms, including antagonistic interactions between the penis and the opening to the female pregenital chamber (Burns et al. 2013). Recent work indicates that the relative maximum forces produeed by the penis protractor muscle and by the closer of the female genital operculum coevolved and are

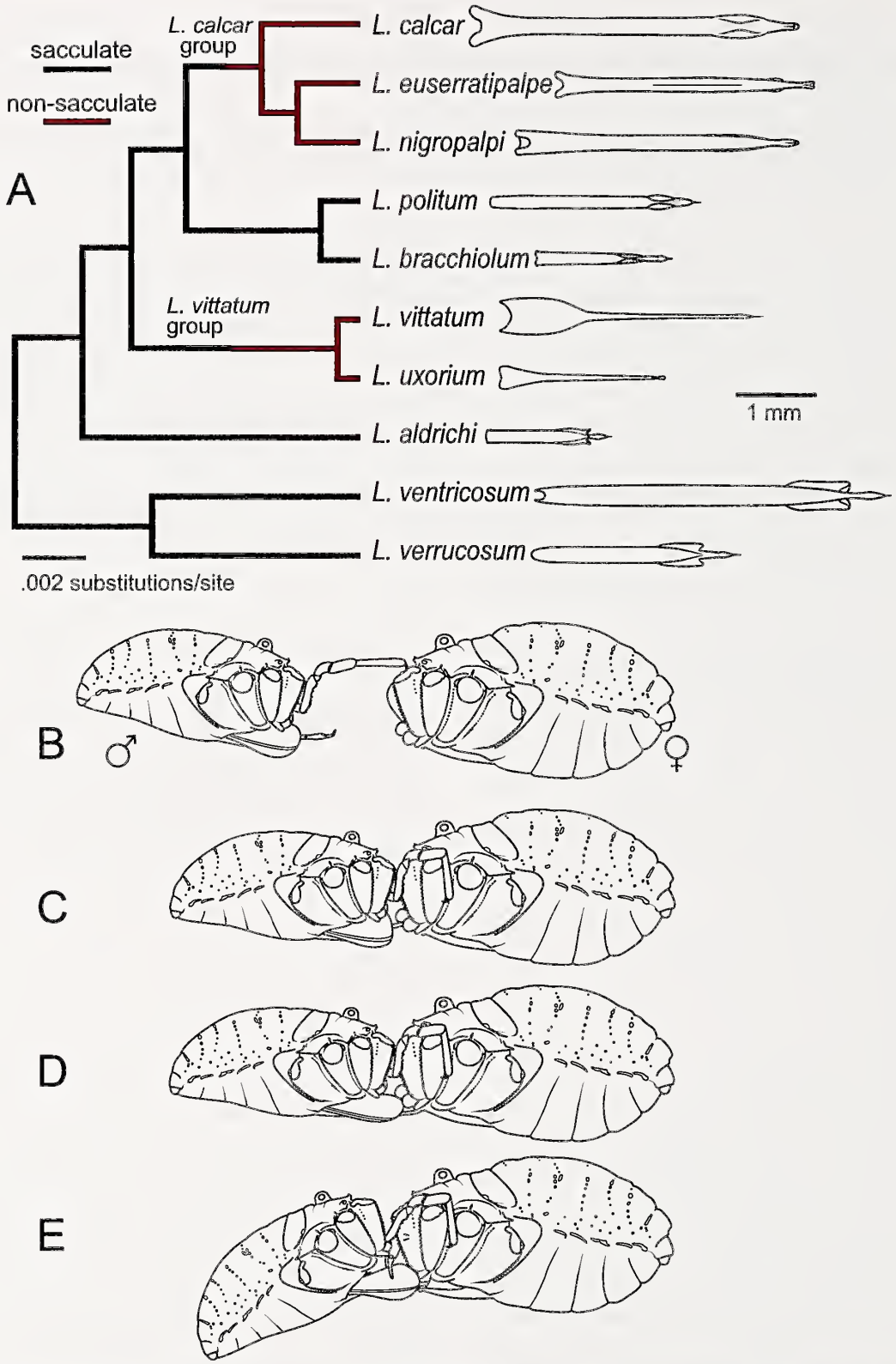

Figure 1.—Male reproductive morphology and phylogeny of *Leiobunum*. A. Penes (to same scale) from 10 *Leiobunum* species depicted on a pruned maximum clade credibility tree (Burns et al. 2013). We hypothesized that these discrete classes should be highly correlated with genital function, such that mechanical force traits might discriminate them. B–E. Mating in *Leiobunum verrucosum* (legs removed for clarity). B. Male encounters receptive female, male palps preparing to grasp female, penis extruded. C. Male clasps female with palps posterior to leg coxa II, delivers initial nuptial gift to female from penile sacs. D. Female feeds from male glands, penis lodged near female pregenital opening. E. Intromission associated with reorientation of bodies.

higher in non-sacculate species, suggesting that greater mechanical forces are produced and resisted in non-sacculate forms (Burns & Shultz 2015).

We hypothesized that the mechanical properties of penes in *Leiobunum* have changed from those that accommodate female preferences to those that can transmit or resist mechanical forces when interacting with the female's pregenital opening. Specifically, we expect penes used in forceful precopulatory interaetions to resist higher bending forces than those used principally for enticing females with nuptial gifts. We also predicted changes in two parameters associated with cuticular viscoelasticity. In structures composed of ideally elastic materials, the energy used in deforming a structure is stored as elastic potential energy and recovered as kinetic energy as the penis regains its resting state, regardless of the duration or rate of loading or unloading (Vincent 2012). However, in viseoelastic materials, some energy is lost to heat during deformation, with the amount being time dependent. Thus, we also predicted that the amount and rate of energy loss would be higher in penes that are adapted to accommodate female nuptial-gift feeding and lower in penes adapted for applying large or prolonged forces to the female

We tested our hypotheses by bending penes from 10 *Leiobunum* species while measuring both flexural resistance and flexural displacement. Measurements were obtained from a phylogenetically diverse sample of five sacculate species and representatives from two non-saceulate clades, the *calcar* and *vittatum* groups (Fig. 1A). We measured three biomechanical variables—maximum resistance force for a flexural displacement of 5% of beam length, the efficiency of elastic energy storage, and the rate of viscoelastic relaxation in static bending. Phylogenetic multivariate analyses of our data recovered two species clusters (the sacculate group and monophyletic *calcar* clade) that were consistent with our biomechanical predictions but also a third cluster that was not anticipated. Ultimately, this work demonstrates that mechanical properties of reproductive structures can be measured and that data derived from these measurements can be used to test hypotheses about arthropod mating systems.

METHODS

Animals.—We examined 60 male specimens representing ten *Leiobunum* species (Fig. 1A), constituting a subset of species examined in Burns & Shultz (2015). Five species have penes with subterminal gift-bearing sacs and females that lack pregenital barriers (i.e., *L. ventricosum* (Wood, 1868), *L. verrucosum* (Wood, 1868), *L. aldrichi* Weed, 1893, *L. politum* (Weed, 1889), *L. bracchiolum* McGhee, 1977), which we term "sacculate species". Five species lack gift-bearing sacs and females have selerotized pregenital barriers (i.e., the *vittatum* group: *L. uxorium* Crosby & Bishop, 1924, *L. vittatum* (Say, 1821) and the *calcar* group: *L. nigropalpi* (Wood, 1868), *L. euserratipalpe* Ingianni, McGhee & Shultz, 2011, *L. calcar* (Wood, 1868)), which we term "non-sacculate species". Speeimens were collected and maintained up to a week in laboratory terraria with food (pulverized fish food) and water provided *ad libitum* in cotton-stoppered vials. See Table 1 for additional species information.

Apparatus.—We assembled a device to measure forces associated with penile flexural resistance and flexural displacement (deflection) in fresh harvestman penes mounted as a cantilever (Fig. 2). We attached a force transducer (Aurora Scientific, Inc.: Model 404A: range, 0-100 mN; sensitivity; 10.0 mN; resolution, 2000 nN) to a vertieally mounted, computer-controlled translation stage (Thor Labs: OptoDC Servo Motor Driver #001). A displaeement transducer (Microstrain: SG-DVRT-4: max. linear stroke, 24 mm; resolution, 6 μm) recorded linear movement of the force transducer. The translation stage was programmed to move at 1 mm/s. A stiff, hooked, non-magnetic wire was attached to the input tube of the force transducer and used in deflecting the penis.

Protocol.—Adult specimens were sacrifieed by plaeing them in a freezer at –20°C for 10 minutes, after which the penis was removed and affixed at its proximal end to a round glass cover slip using ethyl cyanoacrylate gel (Super Glue®). A drop of accelerant (Turbo Set I, Palm Labs Adhesives, Inc.) was applied to the glue bead to fix the penis as a full-moment cantilever (Fig. 2B). The cover slip with attached penis was submerged in a clear-sided, open-top polyacrylic box filled with room-temperature Ringer's solution. The cover slip was affixed to the side of the box using 1/8 inch x 1/8 inch (.3175 cm × 3175 em) neodymium magnets to facilitate repositioning (Fig. 2A).

The hooked wire from the force transducer was brought into contact with the penis at a point one third of free-penis length from the distal end. This length was selected to avoid applying force to the sac region of penes from sacculate species (Fig. 1A). The translation stage was programmed to bend the penis with a deflection of 5% of the beam length. Three consecutive hysteresis loops (Fig. 3A) were obtained from each penis, with the penis displaced and returned to its resting position at 1 mm/s. Hysteresis loops were obtained for dorsal, ventral and horizontal deflection from the resting position, as each of these flexural directions may be imposed by the female pregenital chamber upon the penis during precopulation. We also deflected each penis to 5% of beam length and held it for at least 180 s while measuring the viscoelastic relaxation of the restoring force (Fig. 3B). Displacement and force data were sampled every 50 ms using Easylogger Dual Version 1.0 software (EasySync Ltd.).

Mechanical variables.—We calculated three mechanical variables each from dorsal, ventral and lateral flexures—force at maximum experimental displacement F_{max} (or cF_{max} when correeted for body size), elastic efficieney R, and relaxation time to 90% F_{max} (T_{90})—resulting in a total of nine variables. Mean values for all variables were established for each specimen from three replicates, and mean species values were calculated from specimen means.

F_{max} is the force required to achieve a vertical deflection of the penis equal to 5% of beam length. Consequently, F_{max} can be viewed a measure of stiffness at a geometrically similar flexural displacement across specimens. To adjust the magnitude of F_{max} for body size, we derived a method of size correetion from the standard equation for cantilevered beams fixed at one end (Vogel 2003):

$$FD^{-1} = 3EIL^{-3} \quad (1)$$

and its proportional representation:

$$[FD^{-1}] = EIL^{-3} \quad (2)$$

Table 1.—Taxon sampling for molecular phylogenetic reconstruction and mechanical force trait evaluation. Accession numbers are for the GenBank genetic sequence repository; numbers GQ870643–GQ870668 and GQ872152–GQ872185 are derived from Hedin et al. (2010). Column 5: penile nuptial gift sac presence, grouping variable used in testing for trait mean differences. Column 6: numbers of male specimens analyzed for mechanical force traits.

Species	GenBank accession numbers	Molecular specimen locality	Mechanical data specimen locality	Penile nuptial gift sac presence	Number of specimens
Leiobunum aldrichi	GQ870650, JQ432342, JQ432284, GQ872154, GQ870649, JQ432343, JQ432285, GQ872153, JQ432344, JQ432286, JQ432238	USA: MI: Calhoun Co.	USA: MD: Frederick Co.	Present	2
L. bracchiolum	JQ432330, JQ432272, JQ432230	USA: NC: Guilford Co.	USA: MD: Montgomery Co.	Present	2
L. calcar	GQ870653, JQ432316, JQ432258, GQ872157, JQ432317, JQ432259, JQ432223, JQ432319, JQ432261, GQ870655, JQ432320, JQ432262, GQ872158, JQ432318, JQ432260	USA: MD: Frederick Co.	USA: TN: Carter Co.	Absent	9
L. euserratipalpe	JQ432321, JQ432263, GQ870656, JQ432322, JQ432264, GQ872160	USA: MD: Montgomery Co.	USA: MD: Frederick Co., USA: MD: Montgomery Co.	Absent	10
L. nigropalpi	JQ432323, JQ432265, JQ432224, JQ432324, JQ432266, JQ432225, JQ432325, JQ432267, JQ432226	USA: MD: Frederick Co.	USA: MD: Montgomery Co., USA: TN: Washington Co., USA: VA: Fairfax Co.	Absent	6
L. politum	JQ432326, JQ432268, JQ432227, JQ432327, JQ432269, JQ432228, JQ432328, JQ432270, JQ432229, JQ432329, JQ432271	USA: AR: Lawrence Co.	USA: MD: Montgomery Co.	Present	6
L. uxorium	JQ432339, JQ432281, JQ432235, JQ432338, JQ432280, JQ432236	USA: VA: Smythe Co.	USA: MD: Montgomery Co.	Absent	8
L. ventricosum	JQ432348, JQ432290, JQ432349, JQ432291, JQ432242, JQ432350, JQ432292, JQ432243	USA: TN: Blount Co.	USA: TN: Washington Co.	Present	5
L. verrucosum	JQ432351, JQ432293, JQ432244, JQ432347, JQ432289, JQ432241	USA: TN: Cumberland Co.	USA: MD: Montgomery Co., USA: TN: Washington Co.	Present	3
L. vittatum	JQ432333, JQ432275, JQ432232, GQ870651, JQ432334, JQ432276, GQ872155, JQ432335, JQ432277, JQ432233, JQ432336, JQ432278, JQ432234,GQ870652, JQ432337, JQ432279, GQ872156	USA: TN: Davidson Co.	USA: MD: Montgomery Co.	Absent	4

where F is the imposed bending force (in Newtons), D is vertical displacement of the beam at the point where F is applied (in meters), E is the elastic modulus of the material, I is the second moment of area of the beam, and L is the distance (in meters) between the base of the beam and the point where F is applied. E was assumed to be the same in all penes and was treated as a constant. I is a measure of architectural stiffness and reflects the amount and distribution of material around the flexural axis of a beam. It varies in proportion to d^4, where d is a charaeteristic length of an isometric system. We used the width of the propeltidium of the carapace between coxae I and II (d) as the characteristic length; the propeltidium is a single sclerite and is largely unaffected by nutritional or reproductive status of an adult specimen.

To determine the size correction for F, we solved Equation 2 for F by converting all other parameters to d^n by dimensional analysis:

$$[F_i] = DIL^{-3} = d^1 d^4 d^{-3} = d^2 \quad (3)$$

Thus, we size corrected F_{max} by dividing its measured value by the square of carapace width to obtain cF_{max}.

We obtained elastic efficiency R by dividing the area under the unloading portion of the hysteresis loop W_o (i.e., mechanical energy out) by the area under the corresponding loading portion of the loop W_i (i.e., mechanical energy in) (Fig. 3A, C, D). Given our assumptions of isometry and constant E (see above), no size correction for R was required.

T_{90} was the time (in seconds) required for the force of flexural resistance to undergo viscoelastic relaxation to 90% F_{max}. We did not attempt to correct T_{90} for size given the absence of a time dimension in Eq. 1. In cases where 90% F_{max} was not reached after 180 s, 180 s was used as the relaxation time. cF_{max} and T_{90} were log-transformed to minimize heteroscedasticity between dorsal, ventral and lateral bending and between sacculate and non-sacculate groups.

Data analysis.—We used phylogenetic comparative methods in statistical analysis to control for covariance due to shared evolutionary history. We established a phylogenetic framework by pruning a maximum clade credibility tree from a previous Bayesian-likelihood analysis of leiobunine phylogeny

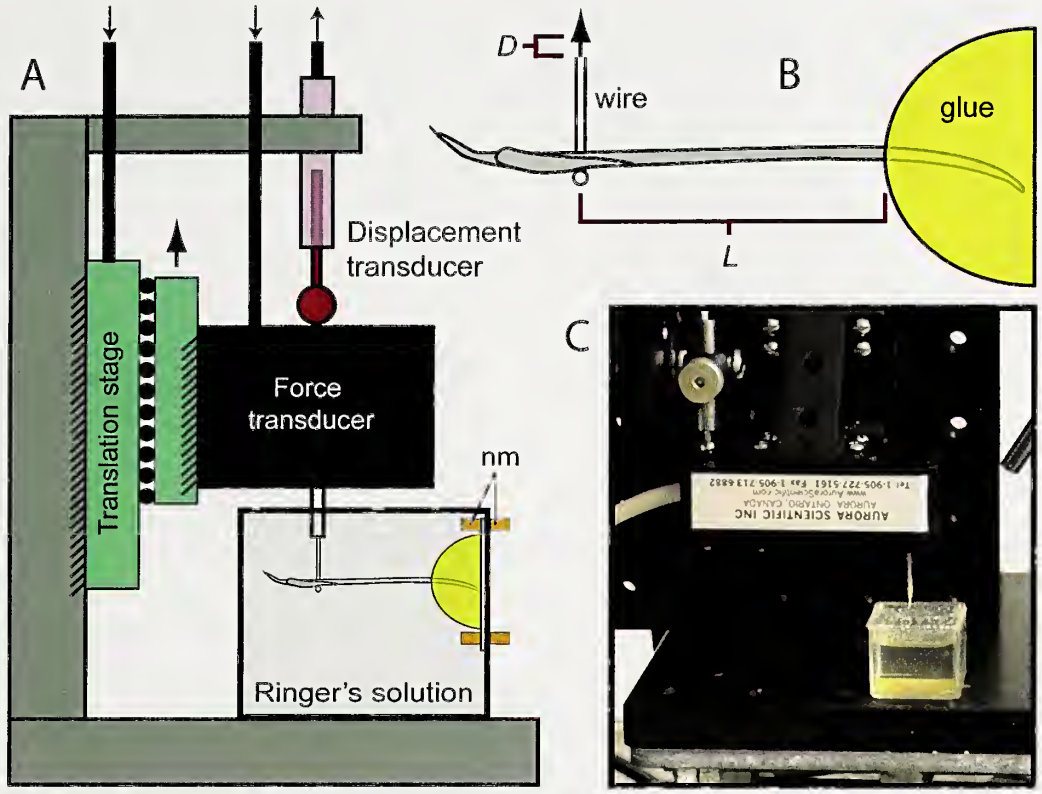

Figure 2.—Experimental apparatus. A. Forces associated with flexural displacement of harvestman penes were measured using a force transducer mounted vertically on a computer-driven translation stage, with vertical movement recorded by a displacement transducer. Penes were glued to a glass coverslip in cantilevered position. The mounted penis was placed in a bath of Ringer's solution and the coverslip was secured to the side of the bath with neodymium magnets (nm). A non-magnetic wire bent at 90° was attached to the input tube of the force transducer, brought into contact with the penis and used to bend the penis in three directions: dorsal, ventral and lateral. B. Lateral view of penis oriented for dorsal flexion. *L*, beam length, *D*, vertical displacement of beam (max. 0.05*L*). C. Photo of apparatus.

(Burns et al. 2013) to include only the 10 species examined here (Fig. 1A). The *geiger* package (Harmon et al. 2008), written in the statistical programming language R (R Development Core Team 2008), was used to evaluate evolutionary models for each variable. The models included Brownian motion, directional evolution (Brownian motion with a trend), Pagel's lambda (phylogenetic signal), kappa (punctuated equilibrium), and delta (time-dependent rates or comparable to early burst evolutionary model) (Pagel 1997, 1999). Evolutionary models were evaluated using the corrected Akaike information criterion (AICc), adjusted by the number of estimated parameters for each model. Model probability was determined by AICc weights (Burnham & Anderson 2004).

We performed phylogenetic principal components analyses using the R-based package *phytools* (Revell 2012) to explore covariation among mechanical variables. The predictions presented in the introduction anticipate positive covariation among variables that should be reflected in similarities in variable loadings and the placement of sacculate and non-sacculate species into separate clusters. Differences in mechanical variables between sacculate and non-sacculate groups were investigated further using phylogenetic multiple analysis of variance (pMANOVA) (Garland et al. 1993) as implemented in the *geiger* package. The Wilk's lambda test statistic and significance level were calculated for the data and for one million Brownian-motion simulations based on the evolutionary variance/covariance matrix estimated from the data across the phylogeny. Thus, model significance indicated by the standard MANOVA is supported by the commonality of the actual-data test statistic compared to a null distribution. We conducted Shapiro-Wilk's and Levene's tests (using the R package *asbio*; Aho 2014) on each variable to assess normality and homoscedasticity.

RESULTS

Models of evolution.—We used the fitContinuous function in the R package *geiger* to determine the best fit based on AICc for five potential models of evolution for each mechanical variable (Table 2). Most variables were best modeled by Brownian motion. This result was reinforced by maximum likelihood estimates of Pagel's lambda, which equaled or approached 1.0 for several traits across the three bending directions, including dorsal $\log T_{90}$, dorsal and lateral $\log cF_{max}$, and ventral and lateral R. A lambda value of 1.0 is considered equivalent to a Brownian motion evolutionary model (Boettiger et al. 2012) and indicates that covariance can be largely attributed to shared evolutionary history (i.e., length of shared branches.) Two mechanical variables had lower AICc scores for non-Brownian models; lateral $\log T_{90}$ was best modeled by the kappa branch transformation ($\kappa =$ 6.6E-214, AICc = 252.56) and lateral R was best modeled by the lambda branch transformation ($\lambda = 0.715$, AICc = 4.06). In both cases, the Brownian model had the next highest AIC

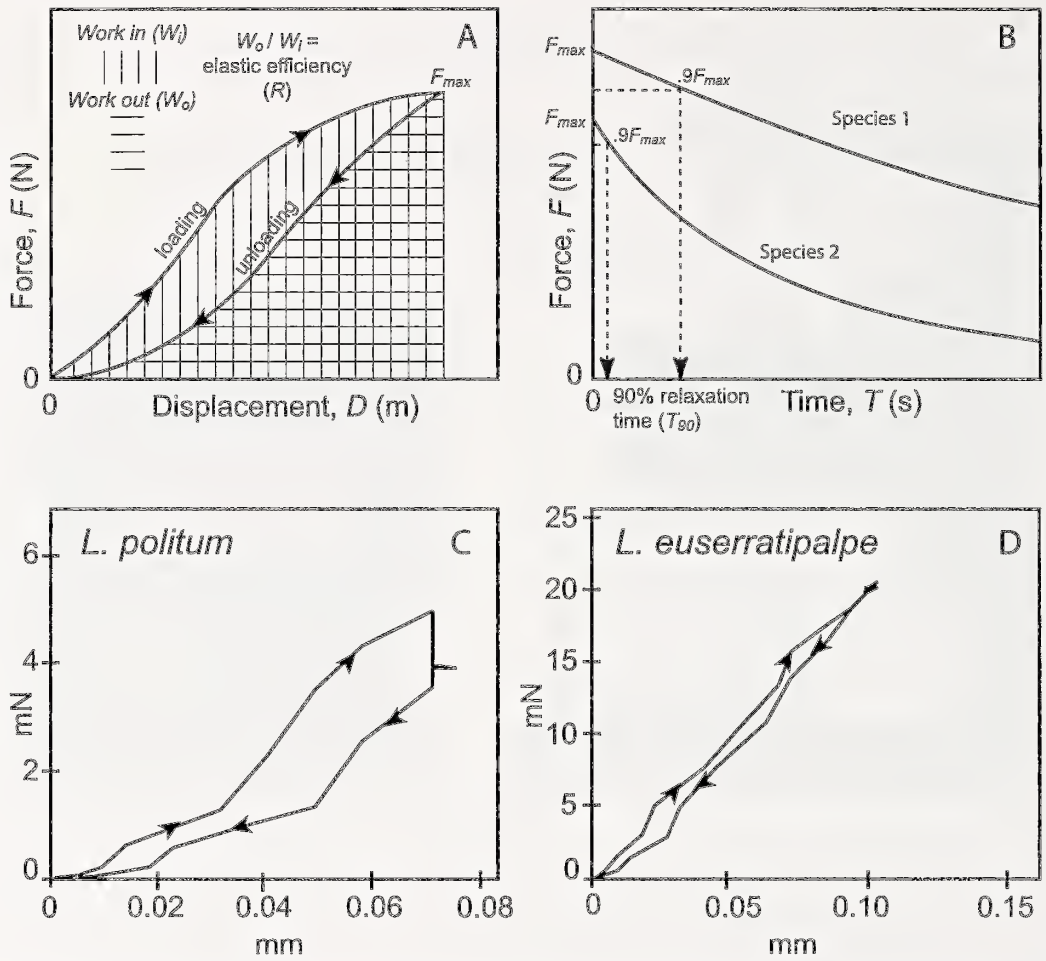

Figure 3.—Diagrammatic plots illustrating biomechanical variables and ventral sample data. A. Hysteresis loop showing parameters used to calculate elastic efficiency *R*. The work generated when a penis is loaded in flexion is proportional to the area under the loading curve (*Wi*). The work generated by the penis against the force transducer during re-extension (W_o) reflects the elastic energy stored in the penis. Thus, W_o/W_i equals *R*. B. Plot illustrating determination of T_{90}, the time required for the maximum force of flexural resistance F_{max} to relax to 90% F_{max}. We anticipated that a penis specialized for coercive interaction with females should have a higher F_{max}, T_{90} and *R* than penes adapted to accommodate female preferences. C. Hysteresis loop for ventral flexion in *Leiobunum politum* specimen (sacculate). D. Hysteresis loop for ventral flexion in a *L. euserratipalpe* specimen (non-sacculate, *calcar* species-group).

weight (lateral $T_{90} = 0.27$; lateral $R = 0.31$), indicating that the alternative model did not provide significant improvement over Brownian motion. However, it may be meaningful that the traits best modeled by the more-complex functions were both derived from lateral bending.

Exploring covariation in mechanical variables using principal components analysis.—We performed a phylogenetic principal components analysis using a multivariate lambda model of evolution to account for phylogenetic covariance due to species relatedness (λ = 6.9E-5, LogL λ = 36.1). Variable loadings are given in Table 3 and plotted alongside species scores in Fig. 4. Principal components 1 and 2 accounted for about 80% of total variance. Several mechanical variables for dorsal and ventral bending loaded highly on PC1, particularly dorsal and ventral logT_{90} and dorsal *R*. Variables associated with lateral bending tended to load more heavily on PC2.

Principal component 1 separated the *L. calcar* group from all other species, indicating that its members had comparatively high dorsal and ventral logT_{90}. Principal component 2 separated four sacculate species (*L. verrucosum, L. aldrichi, L. politum, L. bracchiolum*) into one cluster and the non-sacculate *vittatum* group (*L. vittatum, L. uxorium*) plus the sacculate *L. ventricosum* in another. The *ventricosum/vittatum* group was characterized by comparatively high lateral and ventral *R*, long lateral logT_{90}, and short dorsal and ventral logT_{90}.

Mechanical comparisons between sacculate and non-sacculate penes.—Results from group-mean comparisons using phylogenetic MANOVA are summarized in Fig. 5. No significant difference between sacculate and non-sacculate groups was found for *R* (Wilk's $\lambda = 0.33$, $F_{3,6} = 4.04$, model $P = 0.069$, phylogenetic $P = 0.68$) or logcF_{max} (Wilk's $\lambda = 0.7$, $F_{3,6} = 0.856$, model $P = 0.512$, phylogenetic $P = 0.19$) for any of the three flexural direction. High phylogenetic *P*-values for these models indicate that similar group means were achieved in most simulations, where phylogenetic branch lengths are randomly rescaled to allow greater potential change along longer segments.

There was a significant difference between sacculate and non-sacculate species in logT_{90} (Wilk's $\lambda = 0.26$, $F_{3,6} = 5.77$, model $P < 0.05$) (Fig. 5D), with non-sacculate penes taking

Table 2.—Evolutionary model selection for body size and mechanical force traits. Akaike information criterion corrected for small sample size ($AICc_{wt}$) standardized weights mechanical reproductive traits. Models included Brownian motion (random walk), Directional (Brownian motion with a trend), kappa (punctuated equilibrium), lambda (phylogenetic signal), and delta (time-dependence) (Pagel 1999, 1997). Unstandardized weights were calculated with the equation $AICc_{wt} = e^{((AIC_{minimum} - AIC_i)/2)}$ (Burnham & Anderson 2004). Preferred model (greatest $AICc_{wt}$) is indicated with asterisk (*).

	Model				
Mechanical variable	Brownian ($AICc_{wt}$)	Directional ($AICc_{wt}$)	kappa ($AICc_{wt}$)	lambda ($AICc_{wt}$)	delta ($AICc_{wt}$)
Elastic efficiency (R)					
Dorsal	*0.4098	0.0719	0.1628	0.1553	0.2002
Ventral	*0.5729	0.0768	0.1173	0.1141	0.1189
Lateral	0.3069	0.0528	0.1432	*0.3596	0.1373
90%-relaxation time (T_{90})					
Dorsal	0.6216	0.1962	0.0564	0.0564	0.0694
Ventral	0.5843	0.0851	0.0536	0.1017	0.1752
Lateral	0.2695	0.0453	*0.4407	0.1377	0.1067
Maximum experimental displacement force (F_{max})					
Dorsal	*0.6211	0.0866	0.0882	0.0563	0.1476
Ventral	*0.4059	0.0720	0.1293	0.1742	0.2185
Lateral	0.6525	0.0823	0.0839	0.0592	0.1221

significantly more time to reach 90% of F_{max}. However, this result was not robust to evolutionary data replication (phylogenetie $P = 0.7815$), indicating that a significant group difference based on sac presence would not be found under the majority of $\log T_{90}$ simulations with identical evolutionary conditions. Tests of data normality and heteroscedasticity indicated that all variables were normally distributed, but there were unequal variances between sacculate and non-sacculate species for many variables, primarily those measured during dorsal flexion (dorsal $\log cF_{max}$: $F_{1,8} = 16.7$, $P < 0.01$; $\log T_{90}$: $F_{1,8} = 8.61$, $P < 0.05$). Heteroscedasticity within the non-saeculate category is eonsistent with results from the prineipal components analysis (Fig. 4), where the *vittatum* group tended to cluster with the sacculate species, and not with the non-sacculate *calcar* group.

Although phylogenetic simulation did not identify a significant difference in $\log T_{90}$ between sacculate and non-sacculate species, we performed three follow-up phylogenetic univariate tests comparing means of dorsal, ventral, and lateral $\log T_{90}$. We found significantly longer relaxation times for dorsal ($F_{1,8} = 5.16$, $P < 0.05$) and lateral ($F_{1,8} = 19.21$, $P < 0.001$) bending in non-sacculate species, and a similar, though non-significant, trend for higher ventral $\log T_{90}$ ($F_{1,8} = 0.639$, $P = 0.78$). These results demonstrate significant differentiation of elastic responses in penile cutiele between sacculate and non-sacculate species.

Table 3.—Trait loadings of phylogenetic principal component analyses, eigenvalues, and percent variance explained by first two principal components (PC).

Mechanical variable	PC 1	PC 2
Elastic efficiency (R)		
Dorsal	−0.783	0.254
Ventral	−0.661	−0.655
Lateral	−0.476	−0.811
90% relaxation time (T_{90})		
Dorsal	−0.977	−0.018
Ventral	−0.741	−0.008
Lateral	−0.566	−0.775
Max. resistance force(F_{max})		
Dorsal	−0.726	0.579
Ventral	−0.829	0.452
Lateral	−0.596	0.481
Eigenvalues	4.67	2.52
% Variance	51.89	28.05

Following the result from the phylogenetic principal components analysis, which separated non-sacculate species into *vittatum/ventricosum* and *calcar* groups, we repeated the phylogenetic MANOVA with three group-mean comparisons, separating trait values by sacculate, *vittatum/ventricosum*, and *calcar* group membership. This model yielded significant differences in all three variables (R: Wilk's $\lambda = 0.05$, $F_{6,10} = 5.76$, model $P < 0.01$; $\log cF_{max}$: Wilk's $\lambda = 0.116$, $F_{6,10} = 3.23$, model $P < 0.05$), although in phylogenetic simulation only $\log T_{90}$ was found to be significantly different between groups (Wilk's $\lambda = 0.0079$, $F_{6,10} = 17.107$, model $P < 0.0001$, phylogenetic $P < 0.01$). A phylogenetic ANOVA with Holm-Bonferroni *posthoc* test to compare bending directions for each of the three variables identified signifieantly higher dorsal $\log T_{90}$ ($F_{1,7} = 20.83$, $P < 0.05$) in the *calcar* group as compared to the two other groups, as well as a hierarchy of significant differences in lateral $\log T_{90}$ ($F_{1,7} = 43.52$, $P < 0.01$) between the sacculate, *vittatum/ventricosum,* and *calcar* groups (Fig. 5). Using this method, we additionally found significant differences between the sacculate and *calcar* groups in ventral R ($F_{1,7} = 14.06$, $P < 0.05$) and between the *calcar* and *vittatum* groups in dorsal $\log cF_{max}$ ($F_{1,7} = 18.05$, $P < 0.05$) and ventral $\log cF_{max}$ ($F_{1,7} = 15.18$, $P < 0.05$). Values of R and $\log cF_{max}$ for the *vittatum* + *L. ventricosum* and sacculate groups were statistically indistinguishable.

DISCUSSION

Previous work on the evolution of reproductive structures and mating systems in *Leiobunum* and related taxa (Burns & Shultz 2015) identified the coevolution of relative maximum

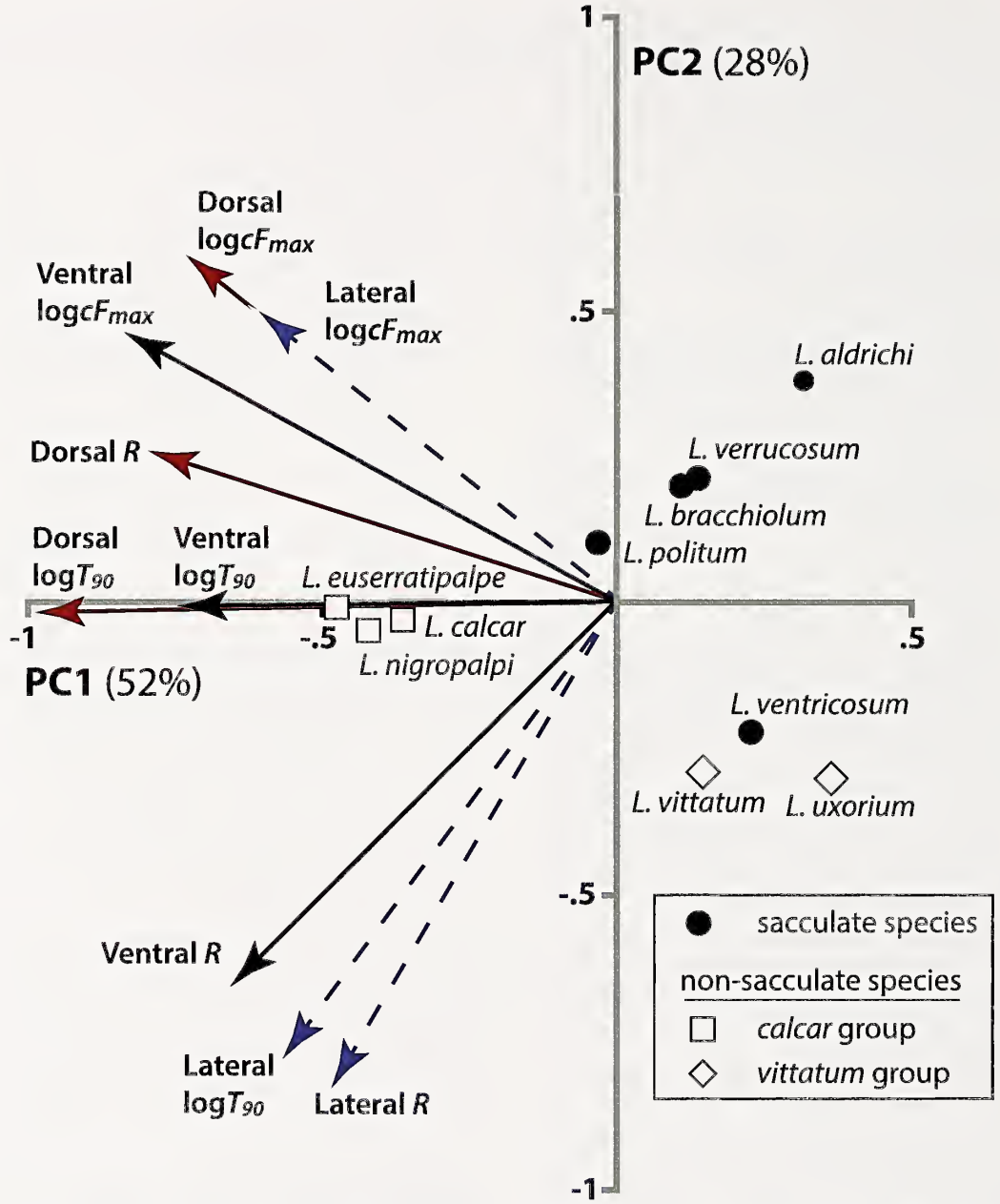

Figure 4.—Phylogenetic principal components analysis of mechanical force data. The pPCA, applied to dorsal, ventral, and lateral measures of maximum flexural resistance (log cF_{max}), elastic efficiency (R) and time to 90% relaxation of F_{max} (logT_{90}). Principal components 1 and 2 together account for 80% of total data variance. Trait loadings are indicated by arrows, color coded by flexural orientation. Non-sacculate species indicated by white shapes—squares for the *calcar* group, diamonds for the *vittatum* group—and sacculate species indicated by black circles.

force produced by the penis protractor muscle and by the closure of the female genital operculum. These relative forces are higher in non-sacculate species, which suggests that greater mechanical forces are produced and resisted in non-sacculate forms. Following this line of evidence, we tested our expectation that presence and absence of penile nuptial gift sacs in *Leiobunum* correspond to differences in the flexural biomechanics of the penis shaft. We predicted that penes in sacculate and non-sacculate species differ in the magnitudes of three biomechanical variables—maximum resistance to experimental penile flexure (F_{max}), efficient storage of elastic energy (R) for use in restoring the flexed penis to the resting state, and persistence of the restoring force during static flexion (T_{90})—with the non-sacculate species having higher values. We obtained these values for each of three biologically relevant bending directions, and used modern phylogenetic comparative methods to find covariation between nuptial gift sac presence and biomechanical specialization. Our success in doing so demonstrates that biomechanical data can be obtained from arthropod genitalia and may be useful in resolving functionally distinct groups.

Results from phylogenetic MANOVA showed no significant differences between sacculate and non-sacculate species groups, except perhaps in T_{90}. These findings are consistent with those obtained from multivariate analysis based on measurements and functional inferences from static reproduc-

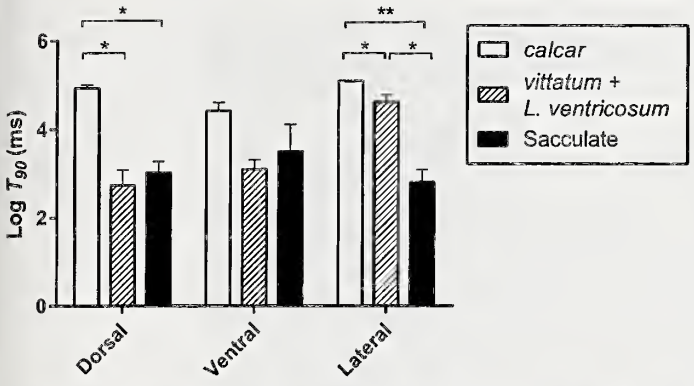

Figure 5.—Phylogenetic ANOVA results for logT_{90}. Bar graph results summarize phylogenetic ANOVA tests comparing the $\log_{10}$-transformed viscoelastic relaxation times of penes displaced dorsally, ventrally, and laterally for sacculate, *vittatum/ventricosum* and *calcar* clusters. Bars are group means plus standard error for sacculate (black), *vittatum/ventricosum* (hatched lines) and *calcar*-group (white) species for each of three bending aspects. Asterisks indicate significance level of between-group comparisons ('*' P_t <0.05; '**' P_t <0.01).

tive morphology (Burns & Shultz 2015). Specifically, canonical correlation, bivariate correlation and principal components analyses placed leiobunine species along a continuum of "antagonistic specialization" in which sacculate species dominated one end and non-sacculate species dominated the other, with a broad region of overlap that precluded classification into distinct sacculate/enticement and non-sacculate/coercion groups. In contrast, results from the current study differ in that pPCA (Fig. 4) appeared to recover three species clusters rather than a single cluster or continuum. PC1 may represent the antagonistic potential associated with dorsal and ventral penile flexure, with higher values toward the left side of the plot. This axis separates the non-sacculate *calcar* group, with high antagonistic potential, from all other species, including the non-sacculate *vittatum* species-group. Furthermore, PC2 appears to correspond to the antagonistic potential associated with lateral flexure and separates the *vittatum* species-group plus the sacculate *L. ventricosum* from the remaining saeculate taxa.

More specifically, PCA results from our current study indicate that penes in the *calcar* group offer greater resistance to dorsal and ventral flexure relative to body size (cF_{max}) than those of the other species examined. Further, relatively more elastic energy is stored in the flexed penis for use in flexural resistance during re-extension (R), and the restoring force persists for a longer time (T_{90}). This result is consistent with initial predictions, but was not found in the *vittatum* group, and the results were thus inconsistent with predictions about non-saceulate penes generally. The species outside the *calcar* group were separated primarily by factors associated with lateral flexure, with saceulate species (except *L. ventricosum*) having greater resistance to lateral flexion relative to body size than the *vittatum/ventricosum* cluster. The relatively greater flexural compliance of the *vittatum/ventricosum* cluster may be attributed to the greater relative length (L) of penes and the low second moment of area (I) of the penis shaft in the *vittatum* group, which is narrow and circular in cross section rather than wide and dorsoventrally compressed as in sacculate species (see Eq. 1; Fig. 1). In contrast, lateral bending in the penes of the *vittatum/ventricosum* cluster showed viscoelastic properties higher than those of most sacculate species (i.e., with higher energy storage and longer relaxation times).

Given the rather low sample size and high experimental variance in some of our data, it is premature to conclude that the three clusters recovered by PCA correspond to functional groups or mating strategies. Furthermore, we modeled displacement assuming penes were the equivalent of a beam, although unlike standard beams, penes of these species are not consistent in width across length. While we did not vary the application site of forces, we could expect incremental changes in second moment of area (I) as the site of displacement (L) is repositioned. However, the combination of mechanical properties within each cluster, together with other morphological and behavioral observations, may offer new insights and testable hypotheses. Specifically, we propose that the *calcar* group still exemplifies an antagonistic mating system in which male coercion and forced penetration have played an important role in shaping genitalic morphology and biomechanics. This conclusion is consistent with the robust pedipalps in males that are used to clasp the female during mating and a sclerotized latch mechanism at the pregenital opening of females (Ingianni et al. 2011). The cluster of four sacculate species may represent a mating system dominated by male enticement of females. Members of this group have short but dorsoventrally flexible penes with gift-bearing sacs. The penes are poorly designed for imposing significant mechanical forces and, in fact, are mounted on a fluid-filled turret (haematodocha) during mating (Fig. 1) that would tend to accommodate rather than resist female movements. In addition, females of the species group have no special elaboration of the pregenital opening that might resist forceful penetration, although larger body size alone may be a sufficient defense. Finally, we speculate that the genitalic features of the mixed *vittatum/ventricosum* cluster could reflect specializations that allow females to coerce prolonged delivery of nuptial gifts from males by entrapping or imposing possible damage to the penis (as in Kuriwada & Kasuya 2011). Such a system would combine elements of enticement by the male and antagonism by the female and might thus account for the intermediacy between enticement and antagonism revealed in our previous study (Burns & Shultz 2015).

A few other lines of evidence are consistent with female coercion of males via penis entrapment. Specifically, recent behavioral analyses of mating in *L. vittatum* from Wisconsin indicate that females may regulate the duration of mating contact, in part, by resisting male attempts at separation (Fowler-Finn et al. 2014) and that males sometimes show signs of physiological exhaustion after mating (K. Fowler-Finn, pers. comm.). Interestingly, male *L. vittatum* from Massachusetts have been found with no other damage than a broken penis (Fig. 6A-C), which is consistent with persistent penile entrapment by the female. Although we previously characterized the sclerotized sterno-opercular mechanism in female *L. vittatum* as a pregenital barrier (Burns et al. 2013; Burns & Shultz 2015), the pregenital mechanism could also be used for entrapping the penis. In contrast, our own video-based observations of mating in *L. ventricosum* show no obvious signs of female coercion during mating. However, *Leiobunum*

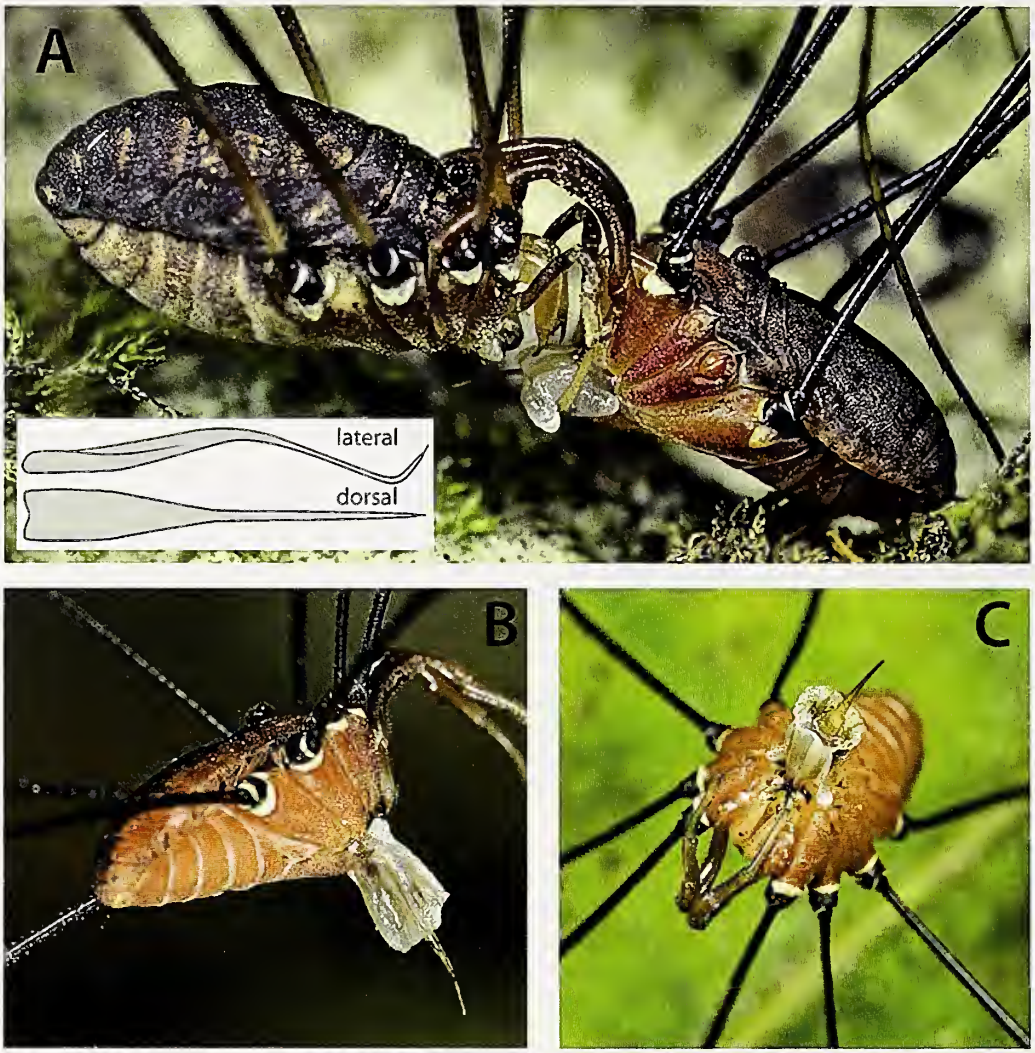

Figure 6.—Male *Leiobunum vittatum* found with broken penes, suggesting mechanical damage incurred during mating. A. Mating *L. vittatum* (female left, male right). Male clasps female coxa II (as in Fig. 1C) with damaged penis extended. Female palpates male haematodocha. Lateral and dorsal drawings of *L. vittatum* penis are inset. B. Ventrolateral view of male *L. vittatum* with broken penis. C. Ventral view of same male. In both examples, male penis is broken at the upturned glans portion. All pictures courtesy of Joe Warfel/Eighth Eye Photography, Massachusetts, USA.

holtae McGhee, 1977 (not included in the present study), a derived species that appears to have evolved from *L. ventricosum*-like ancestors (Burns et al. 2012), is morphologically similar to *L. vittatum* in having a long, thin non-sacculate penis. In addition, the female pregenital opening has a large, horizontally-arranged opercular plate that can be pressed against a large sternite dorsally. Again, this mechanism could be interpreted as either a barrier (which seems substantially overdesigned given the apparent compliance of the penis) or a penis trap. It is therefore possible that the condition in *L. holtae* evolved from a less extreme form of female coercion that may be practiced by *L. ventricosum*. Testing this speculative hypothesis will require new, intensive studies of mating behavior and functional morphology.

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Mating behavior of the solitary neotropical harvestman *Pachyloides thorellii* (Arachnida: Opiliones)

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Abstract. In order to study how sexual selection takes place during mating, it is necessary to have a clear knowledge of the interactions that occur throughout mating and which morphological and behavioral traits are involved. Available information about harvestman reproductive biology is mainly restricted to anecdotal field observations, most of them lacking a detailed description and quantification of mating behavior. In this paper, we study the reproductive behavior of the gonyleptid *Pachyloides thorellii* Holmberg, 1878 (Pachylinae) and provide quantitative and descriptive information about its sexual behavior. We observed 15 matings, measured females and males, and analysed our behavioral data in the context of individuals' sizes. We observed conspicuous pre-copulatory, copulatory and post-copulatory courtship. We also found that females have several strategies to reject males' mating attempts. Like most gonyleptids, males and females of *P. thorellii* mate in face-to-face position; however, we observed that both male and female clasp their chelicerae mutually. This behavior has not previously been reported for the suborder Laniatores. The information obtained through this study establishes the basis for further studies on this species' reproductive biology and supports the suitability of this species as a model to explore the importance of male copulatory courtship for female choice and sperm use.

Keywords: Sexual behavior, pre-copulatory courtship, chelicerae clasp, copulatory courtship

Opiliones is the third most diverse order within arachnids and it is divided into four suborders: Cyphophthalmi, Eupnoi, Dyspnoi, and Laniatores (Pinto-da-Rocha & Giribet 2007). In general, harvestmen are omnivorous, nocturnal creatures, showing high morphological and behavioral diversity (Savory 1938; Coddington et al. 1990; Adis & Harvey 2000). In Cyphophthalmi, reproduction can be achieved asexually through parthenogenesis or sexually through a spermatophore transference (Tsurusaki 1986; Machado & Maeías-Ordóñez 2007). However, the most widespread sperm transfer mechanism in the order is direct copulation. Males possess an eversible penis that is introduced into the females' operculum to achieve sperm transfer (Machado & Macías-Ordóñez 2007).

Although studies regarding harvestman reproductive behavior have significantly increased in the last decade, there are still many gaps in our knowledge (Machado et al. 2015). Most studies on harvestman sexual behavior are field studies on Neotropical species of the suborder Laniatores, particularly of Gonyleptidae, with some kind of parental care. Sexual interactions in harvestmen mainly follow the scheme presented by Machado et al. (2015) where the mating process is divided into three stages: Pre-copulatory, Copulatory and Post-copulatory. In each stage, different sources of selection may shape both the morphology and the behaviors observed in different species (Fowler-Finn et al. 2014). Therefore, having a detailed description of the behaviors observed during these stages is the first step towards understanding sexual selection in each species.

During the Pre-copulatory stage, individuals generally evaluate their partner through courtship and decide whether to continue with further mating (Andersson 1994; Machado et al. 2015). In harvestmen, this stage is brief and courtship involves touching their partner's body (by males, females or both) using legs I and II. There is a small number of species for which courtship has been described; in *Chavesincola inexpectabilis* Soares & Soares, 1946 and *Pseudopucrolia* sp. (Gonyleptidae), the male taps the female's genital opening with legs II and gently touches her dorsum with legs I (Nazareth & Machado 2009, 2010), while in *Zygopachylus albomarginis* Chamberlin, 1925 (Manaosbiidae) it is the female that initially taps the male carapace and legs, and if the male returns the taps then copulation takes place (Mora 1990).

The Copulatory stage involves a closer evaluation of the partner, intromission, and sperm transfer. Stimulation through copulatory courtship is generally the most extended way in which such evaluation occurs (Eberhard 1996; Machado et al. 2015). Copulatory courtship is generally performed by males and consists of touching or grazing the legs and/or dorsum of the female. Males of *Discocyrtus pectinifemur* Mello-Leitão, 1937 and *C. inexpectabilis* (Gonyleptidae) tap females' bodies with legs II and I, respectively, during intromission. In other gonyleptid species such as *Acutisoma longipes* (Roewer, 1913), males intensively tap the dorsum and hind legs of females (Machado & Maeías-Ordóñez 2007; Nazareth & Machado 2009).

Finally, mating is followed by a mate guarding period (Post-copulatory stage). In this stage, the male remains with the female and continues to court and/or stimulate her in order to reduce sperm competition and increase reproductive success (Simmons 2001; Machado et al. 2015). In some harvestman species, males remain with the female, touching her from time to time with legs I and II, until she lays one or more eggs. This period can range from a few minutes (*Z. albomarginis* (Mora 1990); *Iporongaia pustulosa* Mello-Leitão, 1935 (Requena & Machado 2014)) to several days (*A. longipes* (Machado & Olivera 1998); *C. inexpectabilis* (Nazareth & Machado 2009)).

The goal of the present study was to describe the mating behavior of the gonyleptid *Pachyloides thorellii* Holmberg, 1878. This is the first mating description for a solitary species lacking any kind of post-oviposition parental care. We first provide information about the behaviors that conform to the Pre-copulatory, Copulatory and Post-copulatory stages with a

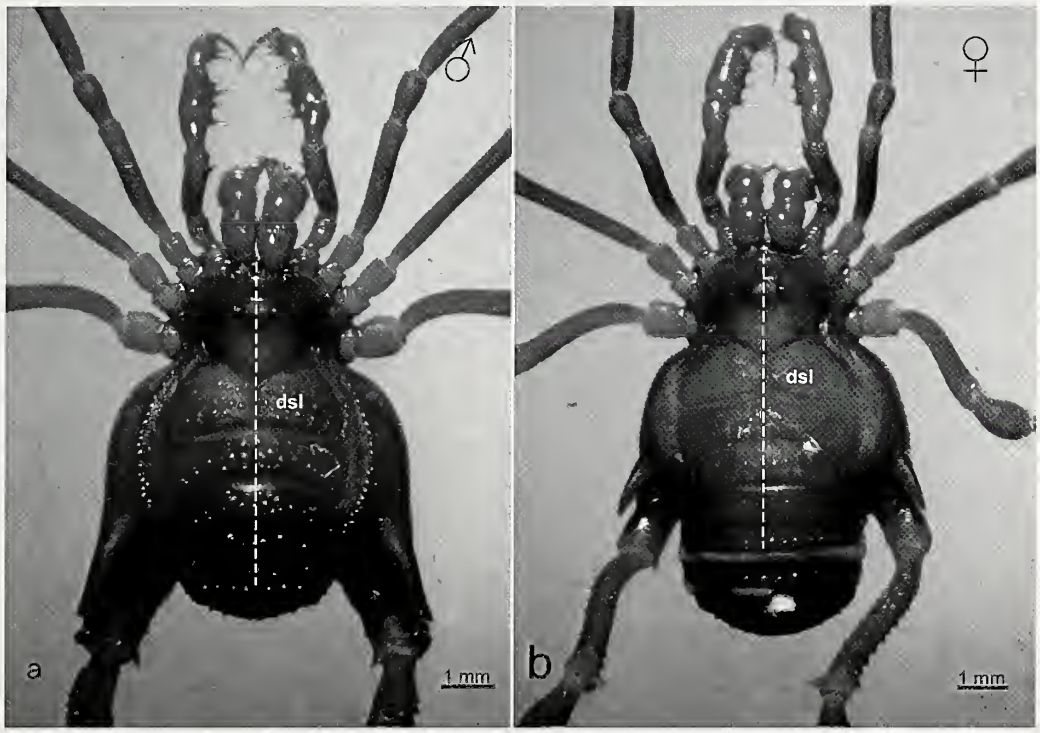

Figure 1.—Measure taken for males (a) and females (b) of *P. thorellii*. dsl: dorsal scute length.

flow diagram. Second, we examine relative sizes of the sexes in the context of the observed behaviors to evaluate whether body size affects mating duration. And finally we provide, for the first time, detailed photographs of the genitalia of this species.

METHODS

Study organisms.—*Pachyloides thorellii* inhabits cryptozoic environments in southern Uruguay, characterized by the presence of leaf litter and pieces of tree bark. Males and females have similar sizes and, contrary to what is observed in other species of the family, they do not seem to be sexually dimorphic (Pinto-da-Rocha & Giribet 2007; Giuliani 2008; Willemart et al. 2008). Females start ovipositing approximately a month after copulation and perform several ovipositions in which each egg is placed in isolation under a rock or inside a tree bark fissure (Stanley 2011).

Collection and maintenance in captivity.—We collected adult individuals of *P. thorellii* at Marindia (Canelones, Uruguay; 34°46′S, 55°49′W), during January 2008 and 2009. The individuals were taken to the Laboratorio de Etología, Ecología y Evolución (I.I.B.C.E., Montevideo, Uruguay) and held individually in Petri dishes of 9 cm diameter and 1.5 cm height, with sand as substrate and wet cotton wool as a water supply. They were fed *ad libitum* once a week with apple and cucumber pieces, cat food, and pieces of *Tenebrio molitor* (Coleoptera) larvae. We maintained individuals under natural photoperiod. The average temperature during breeding was 26.3 °C (± 1.8 SD, range = 17.5–37).

Behavioral observations.—The experiments were performed in March 2008 and 2009, with a room temperature of 24 °C (± 1.2 SD, range = 20–30). Females were placed in Petri dishes of 14.5 cm diameter and 2.5 cm height (encounter arena), with sand as substrate and wet cotton wool to maintain humidity, 24–48 h before the experiments, for acclimation and stress reduction. Males were placed inside the arena immediately before the beginning of each encounter. Males were carefully picked up with forceps by one of their legs IV, to prevent the release of chemical substances that could affect behavior. Then they were gently placed approximately at 10 cm from the female. Each trial lasted 30 minutes after the introduction of the male or until the end of mating. If mating was not observed, the same couple was tested again 24 h later.

All the observations took place under red light (placed 50 cm from the arena). We recorded each encounter with a Sony Handycam video camera (DCR-SR40 Nightshot; Sony Corp., Tokyo, Japan) and took notes of all interactions. We analyzed the video recordings with JWatcher computer program (Version 0.9, Blumstein et al. 2000), to determine the frequency and duration of each behavior. We used the frequency of transition from one behavior to the other and expressed them in percentages to construct the flow diagram.

Morphological features.—After the trials, individuals were fixed and preserved in ethanol 95%. Both males and females were photographed with a digital camera (Nikon Coolpix 5100) mounted on a stereoscopie microscope (Nikon SMZ-10; Nikon Corp., Tokyo, Japan). We took three separate pictures per individual and using ImageTool software (Version 3.0; Wilcox et al. 1995) software, we measured the length of dorsal scute (Fig. 1). We analyzed the average of the measures taken from the three pictures. Following Willemart et al. (2008), we used dorsal scute length as a size reference to calculate an index of size difference for each couple (dorsal scute length of male divided by dorsal scute length of female). This index was related to mating duration in a linear regression, transforming

Table 1.—Description of *P. thorellii* mating behavior units observed. Rejection units were only performed by females.

Behavior	Category	Description
Touch with leg II	Pre-copulatory	Mutual touches with the tarsus of the second pair of legs. The individuals stand still on the substrate touching dorsum, sides and/or first three pair of legs of the partner.
Touch with leg I and II	Pre-copulatory	Male intensively taps female's dorsum with the tarsus of the first pair of legs, while Touches with leg II continues.
Rush	Pre-copulatory	Male extends its pedipalps and quickly approaches the female.
Male over female	Copulatory	Male climbs over female's dorsum and slides over it while he extends its pedipalps and grazes female's dorsum. Touch with leg I and II continues and this behavior ends when the male locates himself in front of her in a face-to-face position.
Grabbing	Copulatory	Once in face-to-face position the male uses the claw of his pedipalps to grab the coxae of the female's first pair of legs. Both grab each other's chelicerae (Fig. 5). Male continues Touch with leg I and II.
Elevation	Copulatory	Using his fourth pair of legs as support, the male elevates the anterior part of his body together with the anterior part of the female's body forming a 90° angle between them.
Copulatory courtship	Copulatory	Male puts the tarsus of both legs I in the dorsum of the female and slides them towards the sides of her body. He maintains his second pair of legs in the air alternating between right and left to touch the female on the sides and dorsum.
Pulls	Copulatory	Female pulls backwards from the male using her third and fourth pair of legs as support.
Leg II movements	Copulatory	Female moves the second pair of legs slowly.
Lowers body	Copulatory	Female bends her legs lowering her body.
Separation	Post-copulatory	Male retracts penis and releases female's pedipalp and chelicerae as the female releases male's chelicerae.
Operculum Cleaning	Post-copulatory	Female scraps the operculum with the claws of her pedipalps and takes them to her mouth. She repeats this several times.
Leg Cleaning	Post-copulatory	Individuals slide their legs through their chelicerae.
End	Post-copulatory	One or both individuals move far away from the other.
		Rejection units
Run away	Pre-copulatory	Female quickly moves away from the male when he touches her.
Bending	Pre-copulatory	Female retracts legs I, II and pedipalps towards her body while elevating the abdomen and lowering the cephalothorax to the substrate.
Kicking	Pre-copulatory	The female rapidly extends leg IV when male approaches.

each variable into logarithmic values. Voucher specimens were deposited in the Colección Entomológica de la Facultad de Ciencias, Montevideo, Uruguay.

Scanning electron microscope (Jeol JSM 5900LV) images were used to visualize the structures present on the penis and the ovipositor of *P. thorellii* individuals. Samples were critical point dried and sputter coated with gold, and scanned at the Servicio de Microscopía Electrónica de Barrido y Micro-análisis, Facultad de Ciencias, Montevideo, Uruguay.

Statistical analysis.—All statistical analysis was performed using PAST (Version 1.18, Hammer et al. 2003).We selected $P = 0.05$ as the limit for statistical significance. We tested the behavioral and morphological data for normality and homogeneity of variances using a Shapiro-Wilk test and Levene test, respectively. If variables showed normality and homogeneity of variances, we used the parametric Student's *t*-test; if any of these conditions was not met we used the non-parametric Mann-Whitney *U*-test. We compared dorsal scute length between males and females to determine whether there was any size difference. Then we performed a multiple regression test using size differences within couples as the independent variable and the duration of the different stages defined during mating as the dependent variable. Finally, we performed a multiple logistic regression using presence and absence of rejection as the categorical variable and mating duration and size differences within couples as continuous variables.

RESULTS

Sexual behavior.—The average duration of the analyzed mating sequences was 690 seconds (± 198 SD, range = 486–1182 s, $n = 15$). We defined 14 behaviors (see Table 1 for description) and displayed the transitions from one behavior to the other in a flow diagram (Fig. 2). The mating process was divided into the three stages proposed by Machado et al. (2015): Pre-copulatory, Copulatory and Post-copulatory.

Pre-copulatory behavior.—Interactions between male and female began when one or both individuals waved their second pair of legs simultaneously or alternately while they remained still or walked around the arena. Contact was initiated by the male in 87% ($n = 13$) of the cases, by directing his second pair of legs and walking towards the female. In the remaining cases (13%, $n = 2$), females initiated contact in a similar way as the majority of males had. When they were close to each other, both male and female touched each other's dorsum and legs with the tarsi of their first and second pair of legs. The Pre-copulatory Behavior stage showed a mean duration of 18 s (± 12 SD, range = 2–48 s). This stage began with the behavior *Touch with leg I and II* and ended with the behavior *Grabbing*. The male touched the female with leg I and rapidly climbed over her dorsum (*Rush*). Once over the female, the male touched the female dorsum both with legs I and pedipalps and the touches with leg II accelerated. He immediately slid over the female until reaching a face-to-face position (*Male over*

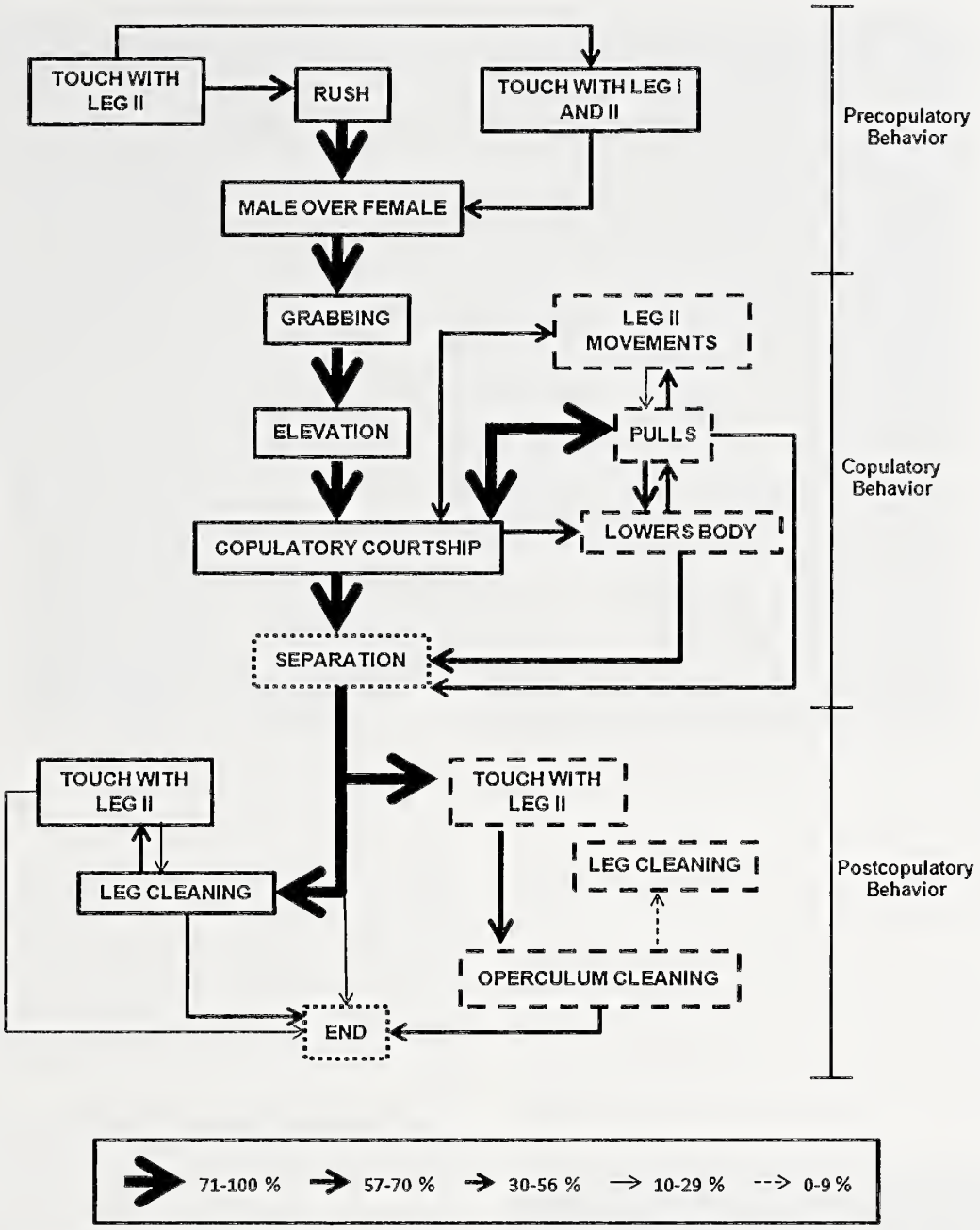

Figure 2.—Flow diagram of *P. thorellii*'s mating. Squares with continuous lines contain behavioral units that were performed only by males. Squares with dashed lines contain behavioral units that were performed only by females, and squares with dotted lines represent behavioral units that were performed by both individuals. Arrow thickness represents different frequencies of transition between units and their value is expressed in percentages.

female). During this behavior, the female was able to reject or offer certain resistance to the male's grabbing attempts. Finally, once in front of the female, the male grabbed the base of her first pair of legs with the claw of his pedipalp and then they both grabbed each other's chelicerae (*Grabbing*) (Fig. 3).

Thirty-three percent ($n = 5$) of the females accepted male courtship and mated without resistance; of the remaining 67% ($n = 10$), 60% ($n = 6$) resisted male attempts to mate but accepted later in the same trial, and 40% ($n = 4$) rejected males but accepted them 24 h later without resistance. The behaviors observed during female resistance and rejections were *Run away*, *Bending* and *Kicking* (see definitions in Table 1). Neither size difference nor mating duration were correlated with the presence and absence of rejection (logistic regression: Size difference: $\chi^2 = 2.1$, $P = 0.15$; Mating duration: $\chi^2 = 0.67$, $P = 0.46$).

Copulatory behavior.—The Copulatory Behavior stage had a mean duration of 654 s (± 152 SD, range = 403–954 s), and started with the behavior *Elevation*. After grabbing the female, the male elevated the front part of his body together with the female, reaching copulatory position (*Elevation;* see Table 1

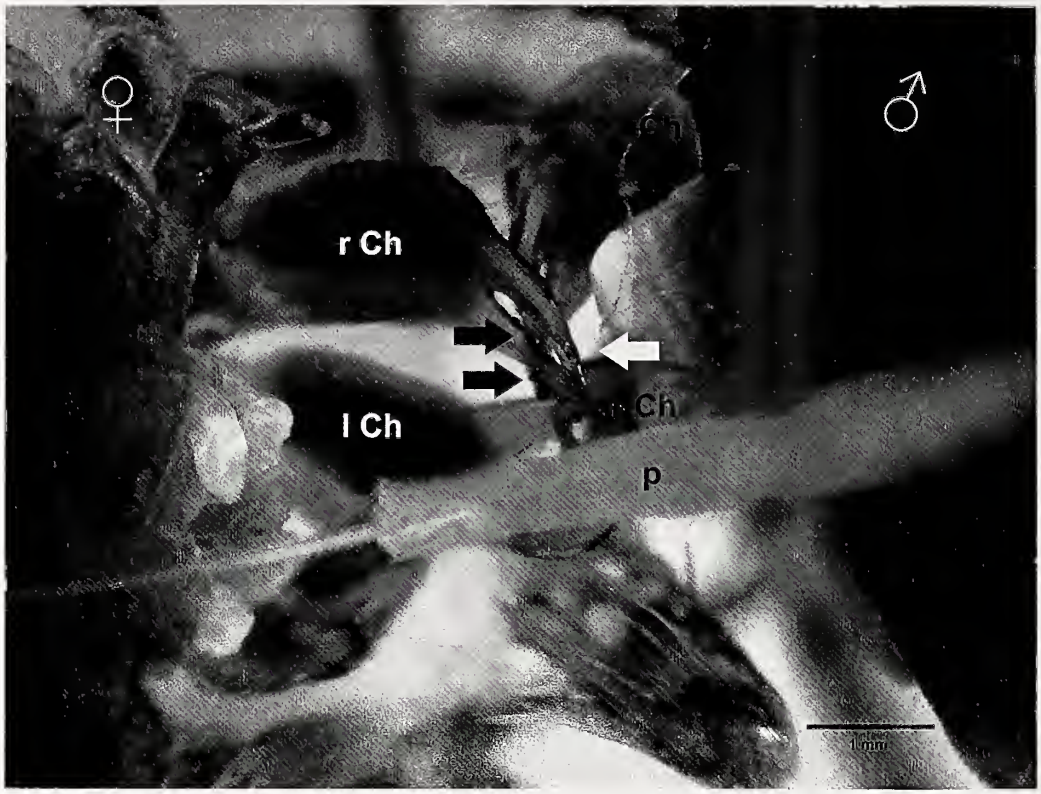

Figure 3.—Ventral detail of mutual cheliceral grabbing during copulation in *P. thorellii*. Black arrows show the sites where male chelicerae grabbed female chelicerae and the white arrow shows female site of grabbing. r Ch: Right chelicerae; l Ch: Left chelicerae; p: penis.

for further detail). Simultaneously, the male raised his third pair of legs until he got them on top of the female's second pair of legs. In this position, while performing *Copulatory courtship*, the male inserted his penis into the female's gonopore and did not withdraw it until the mating ended. At this point, we observed a decrease in the intensity of the male touches with legs I, which then remained constant until the couple separated. Females remained almost immobile during most of this stage, except at the beginning and near the end of the stage when they tried to pull away from the males' grasp. Males maintained their grasp and continued the copulatory courtship. Before *Separation*, females slowly started moving legs II (*Leg II movements*) and lowered their bodies by flexing their fourth pair of legs, which obliged males to withdraw the penis and release the female chelicerae and legs ($n = 11$). Males sometimes finished mating by freeing the female in absence of any of the mentioned female displays ($n = 4$).

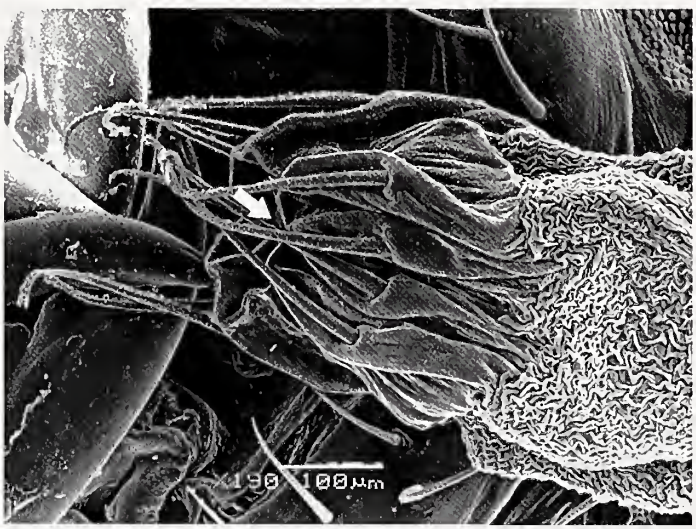

Figure 4.—SEM image of female ovipositor of *P. thorellii*. White arrow shows ovipositor opening.

Post-copulatory behavior.—The Post-copulatory Behavior stage started immediately after the couple separated and had a mean duration of 63 s (± 75 SD, range = 12–258 s). During that stage, both male and female stayed close to each other (approximately 1 cm away), touching each other's dorsum and legs with legs II. At the same time, each of them performed *Leg cleaning*, and during this stage all females were observed carrying out *Operculum cleaning*.

We did not observe a statistically significant relationship between the size ratio of the members of each couple and mating duration or duration of any of the stages (multiple regression: $r = 0.63$, $P = 0.23$).

Genital apparatus.—The female's ovipositor has four lobes, each carrying three long setae that point towards the center of the ovipositor, covering the entrance (Fig. 4). The male's penis has several ornamentations on its *pars distalis* (Fig. 5). We observed on both sides, two groups of three sensilla, one at the distal end and the other on the base (Figure 5a). Between those groups of sensilla, there is a spiny area that covers the edge of the distal part of the penis (Figure 5b). In the *glans*, we observe both the *ventral process* and the *stylus* (Figure 5). In a closer

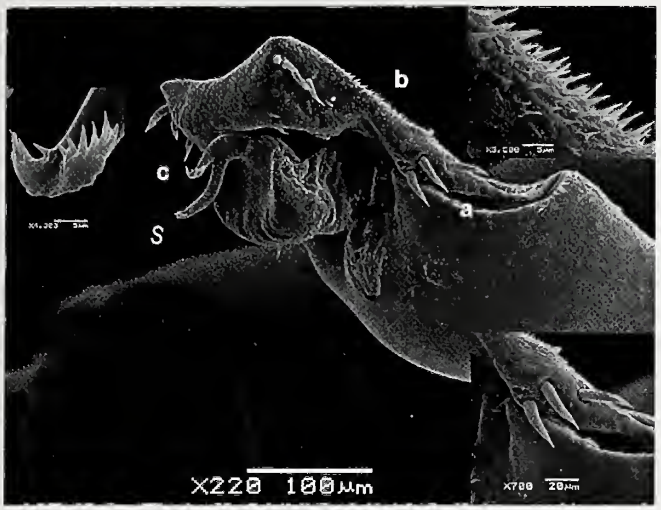

Figure 5.—SEM image of male penis of *P. thorellii*. a, b: detail of sensilla and spines in the *pars distalis*; c: ventral process; *S*: Stylus.

look, the ventral process shows several processes on its sides (Figure 5c).

DISCUSSION

We found that *P. thorellii* mating behavior is one of the longest found so far in the suborder Laniatores, clearly showing the three stages—Pre-copulatory, Copulatory and Post-copulatory—proposed by Machado et al. (2015). Males touched females with legs I and II from the beginning to the end of the interactions and females were able to resist and/or reject males. We observed that females cooperate with the male during copulation, through cheliceral holding and are able to end mating by lowering their bodies, forcing males to withdraw the penis and release their chelicerae. We found no relationship between the size ratio of each couple and either the probability of rejection or the duration of any stage or the whole mating.

As observed in other harvestman species, individuals of *P. thorellii* seem to identify conspecifics and differentiate males from females after touching them (Willemart et al. 2006; Fowler-Finn et al. 2014). Normally, individuals use their second pair of legs to orient themselves to nearby objects; these legs are not used for locomotion and they are constantly moving in a similar way to insect antennae (Machado et al. 2007). As it was observed, prior to contact, individuals direct their second pair of legs towards their conspecific, approach them, and finally contact takes place. Pre-copulatory courtship in *P. thorellii* is similar to that reported for other Laniatores (see Table 12.1 in Machado & Macías-Ordoñez 2007). Particularly in Gonyleptidae, courtship is short and initiates when one individual touches the other. Once the male detects the female, he tries to grab her and mate. However, females can accept mating or resist it. Resistance behaviors in *P. thorellii* resemble those observed in other members of the family (Gnaspini 1995; Elpino-Campos et al. 2001; Machado & Macías-Ordoñez 2007; Willemart et al. 2008; Nazareth & Machado 2009, 2010; Requena & Machado 2014). There was no relationship between rejected males and size ratio within couple members, and rejected males reinitiated courtship several times by touching the female with legs I and II; this behavior may have the function of increasing the probability of female mating acceptance as suggested by Willemart et al. (2006). These facts together with the pre-copulatory behaviors reported by Machado & Macías-Ordóñez (2007) in other species, suggest that Laniatores may rely more on courtship than on coercive behaviors as observed in Eupnoi (Machado & Macías-Ordoñez 2007). The time individuals remain in pre-copulatory courtship represents a window for evaluation of the potential partner and the length of this stage may be correlated with the capacity of the female to control sperm afterwards. It would be necessary to compare courtship duration and the frequency and duration of the behaviors observed during courtship with the number of offspring obtained from virgin females to assess the function of such behaviors.

As mentioned before, the copulatory behavior in *P. thorellii* is one of the longest found so far for the suborder (Matthiesen 1983; Gnaspini 1995; Machado & Oliveira 1998; Elpino-Campos et al. 2001; Machado & Macías-Ordóñez 2007; Nazareth & Machado 2009, 2010; Buzatto et al. 2011) and within species of other suborders (Eupnoi: Macías-Ordóñez 1997, 2000; Willemart et al. 2006; Dyspnoi: Pabst 1953; Martens 1969), and was characterized by tactile courtship (touches with legs I and II) like many other harvestmen (Machado & Macías-Ordóñez 2007). Copulatory position (face-to-face and forming a 90°angle) is similar to what is observed in other harvestmen; the mutual chelicerae holding has not been reported for the suborder Laniatores. Until now it has only been mentioned that females of *Zygopachylus albomarginis* extend their chelicerae and pedipalps and grab males by their cephalothorax to bring them closer, but there has been no mention of male cheliceral holding (Mora 1990). Male cheliceral holding was reported for two species of *Trogulus* Latreille, 1802 (Dyspnoi), in which a male grabs a female's body with legs I and II and her chelicerae with his chelicerae (Pabst 1953). In females, cheliceral holding was observed in a few species of the genus *Ischyropsalis* C.L. Koch, 1839 (Dyspnoi) (see Table 12.1 in Machado & Macías-Ordóñez 2007). Females grab the base of male's chelicerae with her chelicerae, bringing them close to her mouth and maintaining that position until mating ends (Martens 1969). The fact that females actively participate in holding and maintaining mating position suggests they have a greater control of mating duration. In fact, it was observed in these species and both *P. thorellii* and other gonyleptids that females are able to end mating (Pabst 1953; Martens 1969; Nazareth & Machado 2009). *P. thorellii* females lower their bodies, forcing males to withdraw the penis and release the chelicerae. The fact that the male and female hold each other's chelicerae could enable a more firm and stable position during mating and such stability could explain the longer matings observed. Males could use part of the mating time for several purposes: to remove sperm from previous matings (Thomas & Zeh 1984; Eberhard 1996; Birkhead & Møller 1998), to transfer accessory substances (nutritious or inhibitory of future matings) (Parker 1970; Simmons 2001) and/or to stimulate females for longer periods (Eberhard 1998). We found that the penis in *P. thorellii* has spines, sensilla and other projections, such as the ventral process, that could promote penetration of

the penis, remove sperm, and/or stimulate the ovipositor during mating (Macías-Ordóñez et al. 2010). However, the function of these ornaments in this and other harvestman species is still unknown.

After mating, males remain near the female touching her with legs I and II; this fact could indicate the presence of Post-copulatory courtship. A similar behavior was observed in other Gonyleptidae species: in *Chavesincola inexpectabilis*, females oviposit immediately after mating (Nazareth & Machado 2009) and in *Goniosoma spelaeum* (Mello-Leitão, 1933) and *A. longipes*, males stay close and approach to reinseminate the female (Gnaspini 1995; Machado & Olivera 1998). In *P. thorellii*, females oviposit between 30 and 40 days after mating (Stanley & Toscano-Gadea, unpublished data). Males could stay with them for long periods during which they could reinseminate them and protect the female from other males. Even though in this study we separated the couple after they moved away from each other, Stanley (2012) observed that the same couple was able to mate up to six times with a separation of 24–48 h between matings. She also observed that males may fight with one another immediately after matings. These facts could imply that both post-copulatory courtship and mate guarding could be occurring in *P. thorellii*.

Females perform *Operculum cleaning* during most of the Post-copulatory stage. Due to the fact that this behavior is observed immediately after mating it is possible that females are removing and eating sperm (Pinto-da-Rocha & Giribet 2007; Macías-Ordóñez et al. 2010). Females of the fly *Prochyliza xanthosoma* prefer males that transfer great amount of sperm, because after mating they expel part of the ejaculate and feed from it (Bonduriansky & Rowe 2003; Bonduriansky et al. 2005). If females of *P. thorellii* are in fact expelling sperm, this would be one more feature in favor of female control over sperm in this species. Future studies should identify and quantify the substance that the female takes to her mouth and determine if the observed behavior is related with sperm dumping or with other substances with nutritional value being transferred to females as nuptial gifts (Eberhard 1998; Arqnvist & Nilsson 2000; Bonduriansky & Rowe 2003; Elgar et al. 2003; Bonduriansky et al. 2005, Peretti & Eberhard 2009).

P. thorellii seems to be a promising model in which to study the mechanisms responsible for sexual selection. This work provides the framework required for future sexual behavior research in the species. Studies involving courtship influence on mating duration and sperm use in female reproductive tract, as well as the causes promoting male fights, are already taking place.

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The smallest known solifuge: *Vempironiella aguilari*, new genus and species of sun-spider (Solifugae: Mummuciidae) from the coastal desert of Peru

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Abstract. A new genus and species in the South American sun-spider family Mummuciidae, *Vempironiella aguilari* gen. nov., sp. nov., is herein described from a series of specimens from the coastal desert of Punta Hermosa, Peru. *Vempironiella* can be readily distinguished from all other known mummuciid genera, by the absence of the cheliceral movable finger MM tooth and the presence of a diastema between the RFA and RFP teeth on the fixed finger. With this description, the number of valid species of mummuciids is 19, three of which have been described from Peru. Males of *V. aguilari* measure 3.90–5.85 mm in total body length making it the smallest solifuge species known to date. The cheliceral morphology of *V. aguilari* is discussed and some hypotheses on the function of morphology are provided.

Keywords: Solifuges, Punta Hermosa, Peruvian coastal desert.

The South American sun-spider family Mummuciidae Roewer, 1934 encompasses small to moderate-sized species of solifuges. Traditionally, eight genera have been included in the family, namely *Mummucia* Simon, 1879, *Gaucha* Mello-Leitão, 1924, *Metacleobis* Roewer, 1934, *Mummucina* Roewer, 1934, *Mummucipes* Roewer, 1934, *Gauchella* Mello-Leitão, 1937, *Cordobulgida* Mello-Leitão, 1938 and *Uspallata* Mello-Leitão, 1938 (Harvey 2003; Bird et al. 2015). Although the catalogue of Harvey (2003) listed ten genera in the family, two of them, i.e., *Mummuciona* Roewer, 1934 and *Sedna* Muma, 1971, had been transferred to Ammotrechidae by Maury (1982, 1987). Until recently, 20 species were recognized for Mummuciidae (Bird et al. 2015); however, two were discovered to belong to Ammotrechidae (Botero-Trujillo & Iuri 2015).

Mummuciid species have been described mostly from Brazil and Argentina, with six and four species respectively, followed by Paraguay, Chile and Peru, each with two species, and Bolivia and Ecuador, with a single species each (Maury 1998; Xavier & Rocha 2001; Martins et al. 2004; Rocha & Carvalho 2006; Carvalho et al. 2010; González-Reyes & Corronca 2013; Botero-Trujillo & Iuri 2015). These numbers are not accurate estimators of species diversity, however, and enormous areas across the geographical distribution of the family remain unsampled (e.g., Maury 1998: fig. 4). As summarized by Harvey (2003), a few species have been allegedly recorded for more than one country [e.g., *Mummucia variegata* (Gervais, 1849)]. Whilst some of those correspond to rather old records [e.g., Simon's mention of *M. variegata* for Peru (Simon 1879: 152)], determining the actual geographic range of species requires additional dedicated fieldwork and comprehensive efforts to delimit species.

Thus far the recognition of mummuciid genera is a challenging task, for these are poorly defined (Maury 1998; Botero-Trujillo 2014), rendering the validity of some questionable. Because of this, it is often easier to identify new species than it is to place them into a genus. As a consequence, some authors of newly-named species have opted for placing them into the type genus, *Mummucia*, as a conservative approach without taxonomic support (Xavier & Rocha 2001; Martins et al. 2004; Rocha & Carvalho 2006; Carvalho et al. 2010). Two genera with more than one species, *Mummucia* and *Mummucina*, have neither been revised nor had their monophyly yet demonstrated. Meanwhile, the other six genera remain monotypic. Due to this taxonomic confusion, only the study of the type species of the different genera can shed light on where a new species should be placed.

In the present contribution, *Vempironiella* gen. nov. is created to accommodate a remarkable new species, *Vempironiella aguilari* sp. nov., from the coastal desert of the district of Punta Hermosa, Peru. After direct comparison with the type species of the eight former genera of Mummuciidae, the new species proved to exhibit a unique morphology that does not fit into any of the currently recognized genera, all of which are more similar to one another than any is to the new genus. *Vempironiella aguilari* is the smallest known solifuge, with males measuring 3.90–5.85 mm in total body length, with the second smallest being the southern African melanoblossiid *Lawrencega minuta* Wharton, 1981 whose males measure 5–8 mm (Bird et al. 2015).

Vempironiella aguilari represents only the third mummuciid described from Peru, along with *Mummucina exlineae* Mello-Leitão, 1943 and *Mummucina masculina* Lawrence, 1954, and brings the known diversity of the family to 19 species.

METHODS

Terminology used for referring to cheliceral teeth and other cheliceral structures follows Bird et al. (2015). The term *fixed finger retrofondal diastema* (frfd) is here introduced to refer to a toothless diastema present between the RFP and RFA teeth. Abbreviations rlf_{1-4} are here used to identify a set of four individual *principal retrolateral finger* setae, as defined by Bird et al. (2015: 173). These *rlf* setae, which are common to all mummuciid species and are present in at least some other families (e.g., Daesiidae Kraepelin, 1899; see Bird et al. 2015: pl. 145), differ in position across mummuciid taxa (i.e., with respect to particular teeth) and bear some relevant taxonomic usefulness. Identification of individual teeth used the criteria of Bird et al. (2015: 83) for primary homology assessment of

dentition. Leg segmentation terminology follows Shultz (1989). In line with Bird & Wharton (2015), the terms basi- and telotarsus are used for the pedipalp segments traditionally refered to as metatarsus and tarsus. The term 'spiniform setae' (equivalent to spine-like setae) refers to rigid, socketed macrosetae and is preferred over 'spines' (broadly used before by various authors), following recent works on solifuges (e.g., Botero-Trujillo 2014; Bird & Wharton 2015; Botero-Trujillo & Iuri 2015). The formula used to describe the pattern of spiniform setae on telotarsi of legs follows Iuri et al. (2014), where a dash line (-) stands for incomplete segmentation and a slash (/) for complete segmentation. Pedipalp setae terminology follows Cushing & Casto (2012).

The 'row of rigid hairs along the posterior margin of the post-spiracular sternite II' (4th post-genital sternite) is the same structure referred to as 'specialized setae' by Botero-Trujillo (2014) and as 'comb of rigid hairs' by Botero-Trujillo & Iuri (2015). Maury (1984) referred to it as "ctenidia in the form of a comb of rigid hairs". Here the term 'ctenidia' is used only for the long, single-tipped (non-bifid) and flexible seta-like struetures that, in the new species, are present on the 3rd and 4th post-genital sternites. Unlike the rigid hairs which are arranged in a row, ctenidia are irregularly distributed in the sternites (Figs. 22, 23).

The "variation" section deals with observations performed on the cheliceral dental pattern formula and teeth (FSD, FSM) counts (no other significant variation was observed); dental pattern formula follows that proposed by Bird et al. (2015: 67).

Specimens were examined with Leica M165 C and Leica S8AP0 stereomicroscopes. Photographs were taken with a Leiea DFC 290 digital camera mounted on the Leica M165 C stereomicroscope and the extended focal range images composed with Helicon Focus 6.2.2 Pro software (http://www.heliconsoft.com/heliconsoft-products/helicon-focus/). Illustrations of the chelicerae were prepared with CorelDRAW 12 by superimposing veetors on previously obtained micrographs. Images were edited with Adobe Photoshop CS3 (10.0). Measurements, in millimeters, were obtained using an ocular micrometer fitted to a Leitz Wetzlar stereomicroscope.

Some chelicerae were manipulated, after dissection, to allow full display of the dentition. Fine forceps were carefully placed between the finger muera, as close as possible to the bases of FD and MSM teeth. The tips of the forceps were gradually separated by carefully inserting between them the tip of another set of forceps, while controlling the first forceps such that it opened only as desired, i.e., to prevent an abrupt opening that could damage the fingers. Chelieerae were opened enough to expose all the teeth, or until the muscle keeping the movable finger closed had detached. For scanning electron microscope (SEM) preparations, specimens were dissected, cleaned with a fine-bristle paintbrush followed by ultrasonication, dehydrated via 80% - 87% - 96% - 100% ethanol series, fixed to aluminum stubs, and gold-palladium coated in a VG Scientific SC 7620 mini sputter-coater. SEM micrographs were taken under high vacuum with a Philips FEI XL30 TMP.

Material examined.—Specimens used in the present work belong to the following institutions: American Museum of Natural History, New York, U.S.A. (AMNH); Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina (MACN); Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru (MUSM); Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, Brazil (MCN); Museu Nacional do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ); Museum National d'Histoire Naturelle, Paris, France (MNHN); Senckenberg Forschungsinstitut und Natur-Museum, Frankfurt, Germany (SMF).

Specimens of 17 of the other 18 speeies currently placed in Mummuciidae (all but *Mummucia dubia* Badcock, 1932) were examined, including type speeimens of most of them. A list of material examined belonging to the type species of all other genera is provided below.

Cordobulgida bruchi Mello-Leitão, 1938: female holotype (MNRJ): Labels verbatim: "*Cordobulgida bruchi M. L. / Alta Gracia / Bruch leg. / 58160*". "*520 a-D / Leg.: Dr. C. Bruch / Alta Gracia (Cord.) / 14.xii.1934*". ARGENTINA: Córdoba, Alta Gracia, La Granja, under rocks, i.1939, C. Bruch, 2 juveniles (MACN-Ar); Córdoba, Alta Gracia, La Granja, i.1938, C. Bruch, 1 male, 1 female, 1 juvenile (MACN-Ar).

Gaucha fasciata Mello-Leitão, 1924: male holotype (MNRJ, currently at MCN): Label verbatim: "*Gaucha fasciata M. L. / Porto Alegre / Gliesch / 42682*". "*Laboratorio de Zoologia / Solifugos/Solpugidae / Gaucha fasciata / M. Leitão*". 1 male, 2 female paratypes (MNRJ; currently at MCN): Label verbatim: "*Laboratorio de Zoologia / Solifugos/Solpugidae / Gaucha fasciata / M. Leitão*". BRAZIL: Rio Grande do Sul, Porto Alegre, Jardim Botânico, granito, 46 m elev., 30°03′13.11″ S 51°10′35.18″ W, 19.xi.2012, 3 males, 1 female (MCN-Sol-020); 03.xii.2012, 2 males, 2 juveniles (MCN-Sol-021); xii.2014, R. Ott & R. Botero Trujillo, 1 male (96% ethanol, MCN).

Gauchella stoeckeli (Roewer, 1934): 2 males, 1 female syntypes (SMF): Label verbatim: "*Arachn. Coll. Roewer – Lfd. No. 2984 / Solifuga: / No. 73 / Gaucha stoeckeli n. sp. / 2♂, 1♀ / Bolivia, La Paz / Typus / Roewer det. 1933*".

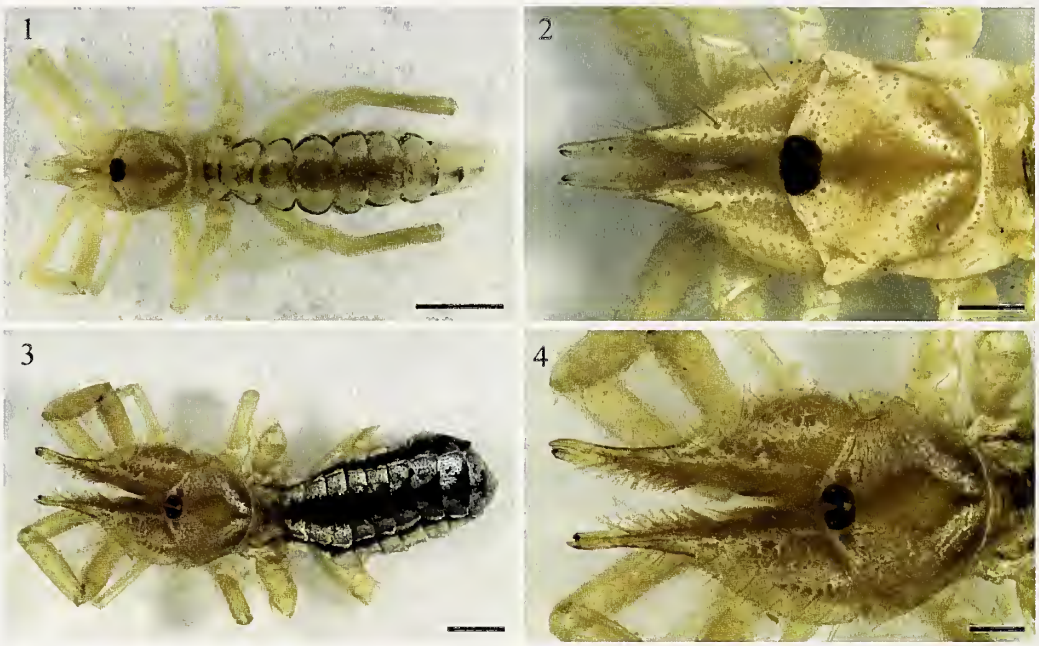
Metacleobis fulvipes Roewer, 1934: male holotype (SMF): Label verbatim: "*Arachn. Coll. Roewer – Lfd. No. 4556 / Solifuga: / No. 365 / Metacleobis fulvipes / 1♂ / n. g. n. sp. / Brasil: Matto Grosso, Cuyabo / Typus / Roewer det. 1933*". "*4756*".

Mummucia variegata (Gervais, 1849): 3 female syntypes (MNHN): Labels verbatim: "*I7849 / Mummucia varegata* [sic] / *Chili / Gervais / Vid. Kraep.*" "*59.*" CHILE: V Región, Valparaíso, Puente Las Bayicas, 24 km E of Algarrobo, 09.xi.1988, E. Maury, 15 males, 1 female, 2 juveniles (MACN-Ar).

Mummucina titschacki Roewer, 1934: ECUADOR: Chimborazo, Road 35th, 3 km N of Riobamba, 1 km before San Andrés, 100 m from "Cantera (quarry) San Andrés", 3000 m elev., 01°35′57″ S 78°41′50″ W, manual eapture and pitfall traps (12:00 to 15:00 hs), 22–23.iii.2014; R. Botero Trujillo, 31 males, 3 females, 4 juveniles (MACN-Ar).

Mummucipes paraguayensis Roewer, 1934: 2 males, 1 female syntypes (SMF): Label verbatim: "*Arachn. Coll. Roewer – Lfd. No. 4753 / Solifuga: / No. 362 / Mummucipes paraguayensis / 2♂, 1♀ / n. g. n. sp. / Paraguay: Asuncion / Typus / Roewer det. 1933*". "*4753*".

Uspallata pulchra Mello-Leitão, 1938: ARGENTINA: Mendoza, Las Heras, 10 km N of Uspallata, 2014 m elev.,



Figures 1–4.—*Vempironiella aguilari* gen. nov., sp. nov. 1–2. Male holotype (MACN-Ar-35453); 1. Habitus, dorsal view; 2. Prosoma, dorsal view. 3–4. Adult female paratype (MACN-Ar-35454); 3. Habitus, dorsal view; 4. Prosoma, dorsal view. Scale bars: 1 mm (Figs. 1, 3); 0.3 mm (Fig. 2); 0.5 mm (Fig. 4).

32°32′30.8″ S 69°18′22.2″ W, manual capture, 22.i.2014; H.A. Iuri, R. Botero Trujillo, A.A. Ojanguren Affilastro, 1 female (96% ethanol, MACN-Ar).

NOTE: Mello-Leitão (1938) only reported one type specimen for *C. bruchi* which was thus far considered lost (Kury & Nogueira 1999). One specimen, accompanied by a label in Mello-Leitão's handwriting and with collection data matching that reported in the original description, was recently found in the collection of the MACN. Although the specimen is not accompanied by any label identifying it as a type, the morphology and wear pattern of its chelicerae (which is very particular) allowed the author to determine that it is, without a doubt, the same specimen illustrated by Mello-Leitão (1938: figs. 72, 73). Therefore, this specimen is considered to be the holotype of *C. bruchi*.

TAXONOMY

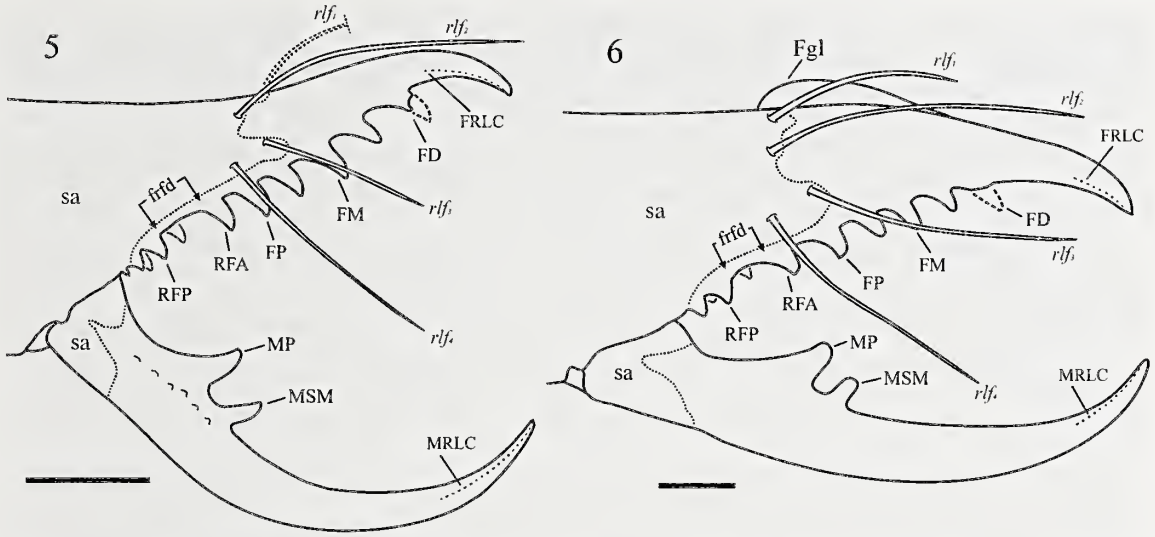
Family Mummuciidae Roewer, 1934
Vempironiella gen. nov.

Type species.—*Vempironiella aguilari* sp. nov.

Etymology.—The generic name is an arbitrary combination of letters that resembles the word "vampire" inspired by the shape of the cheliceral teeth which are reminiscent of the fangs of vampires. Feminine in gender.

Diagnosis.—A member of the family Mummuciidae because of having a three-dark-band pattern on the opisthosomal dorsal surface (Figs. 1, 3), a row of rigid hairs along the posterior margin of post-spiracular sternite II (4th post-genital sternite), lacking spiniform setae on pedipalps (Fig. 16), and the male flagellum of the composite type, retrolaterally compressed with ipsilateral opening, and immovably attached to the cheliceral fixed finger (Figs. 12, 13) (Maury 1984; Bird et al. 2015; Botero-Trujillo & Iuri 2015). The new genus differs from all other genera in the family in various aspects, mostly of its cheliceral morphology: *i)* Fixed finger with retrofondal diastema (frfd) between the RFA tooth and the RFP tooth (intermediate retrofondal teeth absent) (Figs. 5, 6). *ii)* Movable finger with MP and MSM teeth only, MM tooth absent (Figs. 7, 11). *iii)* Movable finger MSM tooth markedly pronounced and columnar (Figs. 7–11). *iv)* Movable finger of female aculeus-like, with very long and slender mucron, and teeth located in a notieeably basal position on the finger (Figs. 5, 7). *v)* Movable finger of female with mucron cylindrical, retrolateral carina obsolete (represented by shallow granules on the base of finger and edge earina on the apex), and gnathal edge carina identified only by a sclerotized line along the mucron dorsal margin (Figs. 5, 7). *vi)* Opisthosomal lateral pleural membranes, sub-dorsal dark bands with white marks surrounding the insertion socket of some setae, instead of similar but black marks on the sub-ventral whitish bands of the membrane.

Comparisons.—All other eight genera currently recognized in the family, most importantly their type species, differ substantially from the above description by: *i)* Cheliceral fixed finger retrofondal teeth series is uninterrupted, without diastema. *ii)* Movable finger with MP, MSM and MM teeth present. *iii)* Movable finger MSM tooth small to moderately pronounced and sub-triangular. *iv)* Movable finger mucron of female moderately long and more robust than that of *Vempironiella*, with teeth located in a sub-medial position on the finger. *v)* Movable finger of female with retrolateral carina moderately to densely granular and gnathal edge carina identified by pronounced angle formed by adjacent surfaces, which gives the appearance of a cutting edge along the mucron. *vi)* Opisthosomal lateral pleural membranes, sub-ventral whitish bands with black marks, and not the other way around, except for *Mummucina* in *stricto sensu* (i.e., *M.*



Figures 5–6.—*Vempironiella aguilari* gen. nov., sp. nov. Schematic representation of the cheliceral morphology in retrolateral aspect. 5. Female and juvenile morphology; 6; Male morphology. Abbreviations: RFP, RFA, FP, FM, FD, particular fixed finger teeth for reference; MP, MSM, movable finger teeth; rlf_{1-4}, set of four principal retrolateral finger setae; Fgl, flagellum; sa, setose areas; MRLC, movable finger retrolateral edge carina; FRLC, fixed finger retrolateral edge carina; frfd, fixed finger retrofondal diastema. Scale bars: 0.25 mm (Fig. 5); 0.1 mm (Fig. 6).

titschacki) which shares the pattern described above for *Vempironiella*.

Vempironiella aguilari sp. nov.
Figures 1–23; Table 1

Mummucia variegata (misidentification): Aguilar 1977: 91 [as "*Mummucia variegata* (?)"].

Type material.—*Holotype male:* PERU: Lima, Lima, Punta Hermosa, "40 km S of Lima", 03.xi.1974, P. Aguilar (MACN-Ar-35453). *Paratypes:* PERU: same data of holotype, 12 males, 2 females, 2 juveniles (MACN-Ar-35454), 1 male, 1 juvenile (AMNH), 1 male, 1 juvenile (MUSM). All specimens preserved in 80% ethanol.

Etymology.—The species is named after the prominent Peruvian Biologist, Dr. Pedro G. Aguilar Fernández (1926–2013). Doctor Aguilar Fernández was the collector of the type material and, in one of his 1977's publications, presented some information about the natural history of this species.

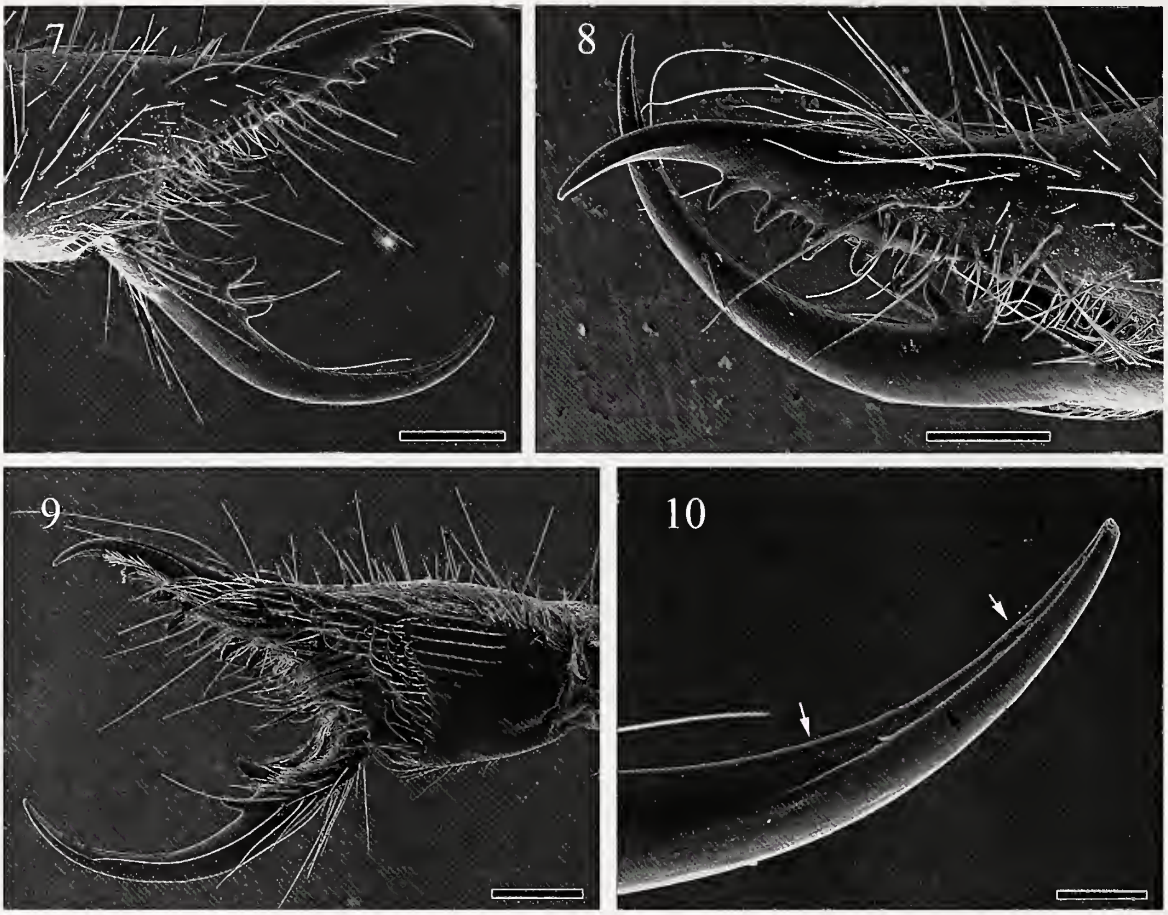
Diagnosis.—As for genus.

Description of male.—Meristic data in Table 1.

Color: (Figs. 1, 2). On 80% ethanol-preserved specimens. General coloration yellow with iridescent white areas. Propeltidium with yellow central area, longer than wide, and two yellow areas on posterior margin, all forming an arrow-like design that is surrounded by white pigment; ocular tubercle yellowish-brown, except for the border of the eyes which is black. Chelicerae manus yellow, ornamented with longitudinal white bands which fuse together on the distalmost region of the setose area; fingers yellow, translucent. Meso-, metapeltidium, and dorsal surface of opisthosoma with a three-dark-band design typical of the family: tergites with median, longitudinal light brown band, and paired lateral white bands; lateral pleural membranes with sub-dorsal dark-brown and sub-ventral white bands; dark bands of opisthosomal pleural membrane with white marks surrounding the insertion socket of some setae; sternites immaculately iridescent white. Ventral aspect of prosoma, legs and pedipalps uniformly yellow, with hint of iridescence; sternum lighter than coxae. Malleoli yellow, translucent.

Prosoma: (Fig. 2). Propeltidium wider than long; with bifurcated setae of variable size, the longest setae arranged in a bilaterally symmetrical distribution on propeltidium; anterior margin procurved, with ocular tubercle elevated; complete and shallow median longitudinal furrow present; anterolateral propeltidial lobes separated from the propeltidium principal shield by incomplete lateral groove. Meso- and metapeltidium wider than long, with bifurcated setae of variable size. Coxae densely covered with bifurcated setae; some of which are longer and exhibit a bilateral symmetrical distribution, and one or two other long single-tipped setae present at least on coxae III. Sternum glabrous.

Chelicera-dentition and processes: (Figs. 6, 11–15). Fixed finger with median teeth series comprising all primary teeth, i.e., FP, FM, FD, markedly pronounced and columnar; secondary teeth arranged in two (FSM and FSD) categories, similar to principal teeth but slightly shorter; retrofondal teeth series comprising RFA, RFP and RFSP teeth only, interrupted by retrofondal diastema (frfd) between the RFA and RFP teeth; RFA and RFP larger than RFSP, both similar to teeth of median series; profondal teeth series with three teeth (PFSP, PFP, PFM); PFM tooth visible in retrolateral aspect through the frfd. Movable finger with median teeth series comprising only two teeth, markedly pronounced and erect MP, and pronounced and columnar MSM, arranged as MP>>MSM; teeth placed in a sub-basal, rather than medial, position on the finger. Movable finger without any trace of MM tooth and without subproximal (MSP) or subterminal (MST) teeth; retrolateral carina incomplete and obsolete, consisting of one or two low granules basal to MP tooth, and keel-like section

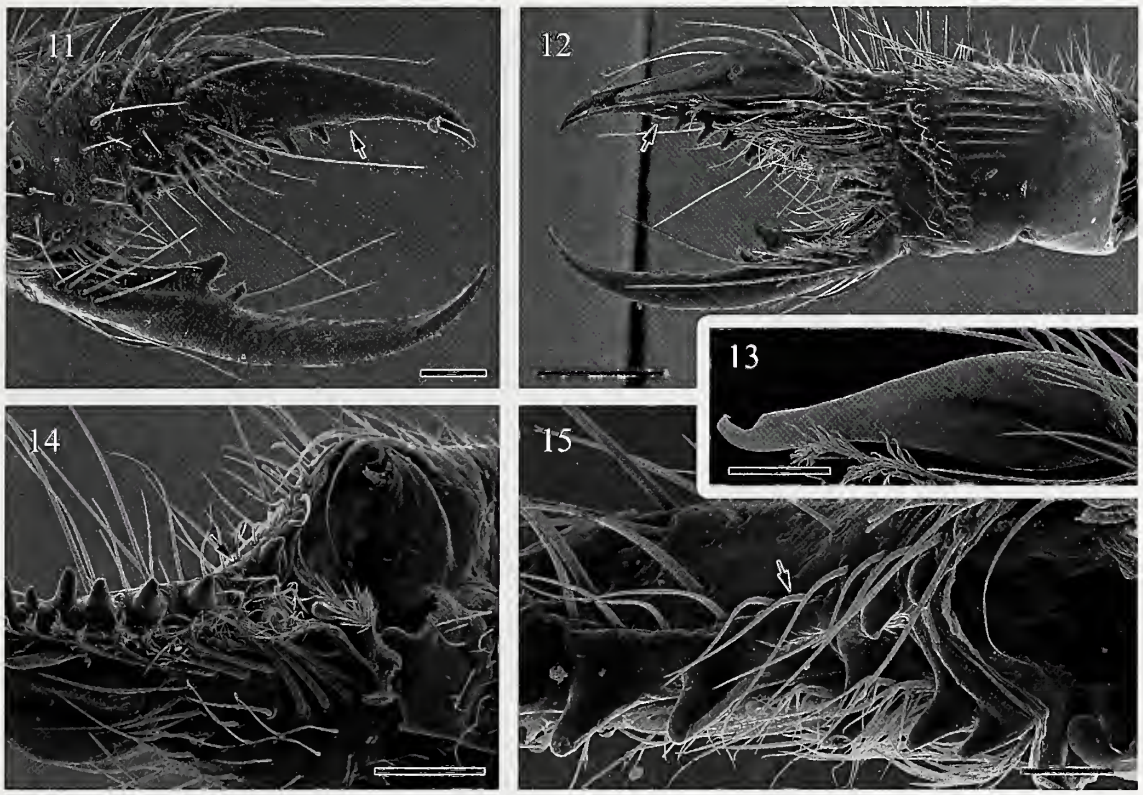


Figures 7–10.—*Vempironiella aguilari* gen. nov., sp. nov. SEM images. Juvenile (presumably subadult) and adult female paratypes (MACN-Ar-35454). 7. Juvenile, right chelicera, retrolateral aspect; 8. Adult female, left chelicera, retrolateral aspect; 9. Juvenile, right chelicera, prolateral aspect; 10. Juvenile, right chelicera, apex of movable finger mucron, retrolateral aspect (gnathal edge carina indicated by white arrows; retrolateral edge carina indicated by black arrow). Scale bars: 0.25 mm (Figs. 7–9); 50μm (Fig. 10).

(i.e., retrolateral edge carina) evident only on the apical region of the mueron. Closure of FP tooth distal to MP. Fixed finger with prodorsal carina complete (along the entire length of the asetose area), starting approximately at level of the attachment point of the flagellum and of RFA tooth, predominantly straight, without angular dorsal crest; proventral carina long, starting approximately at level of FM tooth and present in the entire mucron area; mucron long and slender, ventral margin gently curved, without subterminal flange (STF), apex (FT tooth) ventrally curved. Movable finger mucron very long and slender, with obsolete gnathal edge earina, identified by subtle angle formed by adjacent surfaces.

Chelicera-setose areas and stridulatory plate: (Figs. 6, 11–15). Retrolateral and dorsal surfaces with abundant bifurcated retrolateral manus (*rlm*) and retrolateral finger (*rlf*) setae, of different sizes; some of these setae are arranged in a bilaterally symmetrical pattern, including four evident principal retrolateral finger (*principal rlf*) setae, i.e., rlf_{1-4}, with distribution in the fixed finger as shown in Fig. 6. Prolateral surface with array of setal types, as follows: proventral distal (*pvd*) setae consisting of (apparently) two rows of plumose setae, the ventral reaching the level of the fondal interdigital articular membrane (*fiam*) and the dorsal reaching the prolateral interdigital condyle (*pic*); proventral subdistal setae made up of few thick and blunt setae (*pvsd* eomb) at level of the stridulatory apparatus, and a few others, thinner, in more distal position (*pvsd*); carpet-like field of sparse barbed and bristle-like promedial (*pm*) setae, covering the distalmost quarter of manus. Stridulatory plate slightly longer than high, oecupying most of manus, dorso-apically with a six-ridged stridulatory apparatus (variability in ridge number was not measured). Prolateral setose area of movable finger with setal insertions reaching the level of MP tooth; movable finger prodorsal (*mpd*) setal series consisting of plumose setae arranged in one staggered row or two rows, followed by sparse setae of different length and thickness corresponding to the movable finger promedial (*mpm*) and proventral (*mpv*) setal series, the distalmost setae of each of which is longer.

Flagellum: (Figs. 6, 11–14). A thin, translucent, membranous structure immovably attached prodorsally to the fixed finger; ipsilateral opening present. General aspect drop-like, moderately inflated and narrowing anteriorly; ventral margin sinuous. Visible (prolateral) surface almost smooth, with very sparse minute spicules, barely identifiable along regions of prodorsal margin; apex without visible spicules; apex of the flagellum reaching about midway between the apex of the mucron and FD tooth.



Figures 11–15.—*Vempironiella aguilari* gen. nov., sp. nov. SEM images. Male paratypes (MACN-Ar-35454). 11. Right chelicera, retrolateral aspect (broken FD tooth indicated by arrow); 12. Right chelicera, prolateral aspect (broken FD tooth indicated); 13. Right chelicera, flagellum, prolateral aspect; 14. Left chelicera, fixed finger, proventral aspect (retrofondal diastema indicated by arrow); 15. Ibid., retroventral aspect. Scale bars: 0.1 mm (Figs. 11, 13, 14); 0.25 mm (Fig. 12); 50 μm (Fig. 15).

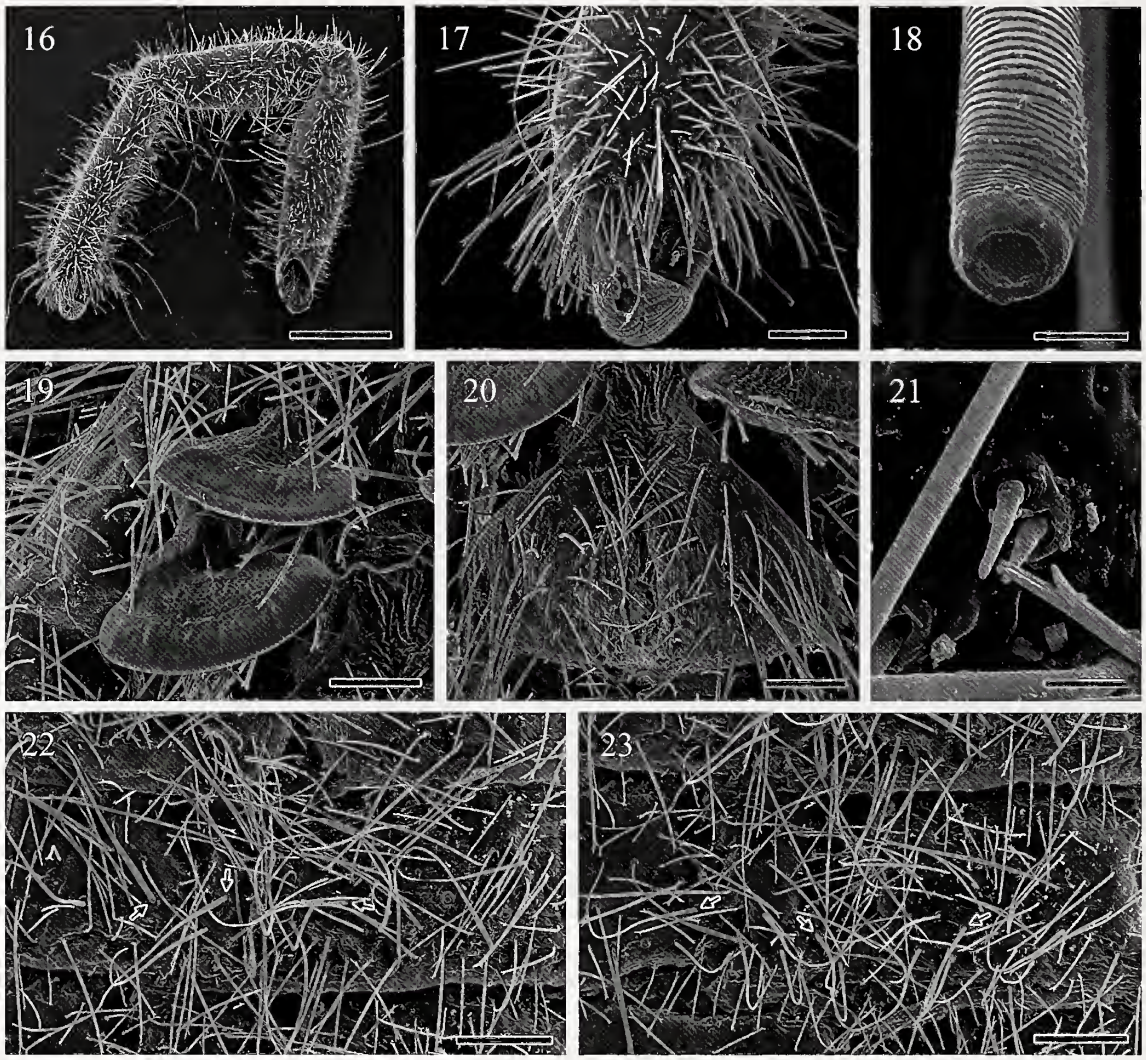
Pedipalp: (Figs. 16–18). Segments robust, all coated with bifurcated setae (*sensu* Cushing & Casto 2012) of different sizes; femur, basitarsus, and especially tibia with ventral set of very long setae, some of them longer than tibia; clubbed setae (*sensu* Cushing & Casto 2012) only present on basi- and telotarsus; spiniform setae absent. Randomly distributed slit sensilla present at least on tibia, basi- and telotarsus.

Leg I: (Fig. 1). Similar to pedipalp with respect to the types, density and distribution of setae; with neither claws nor spiniform setae. Slit sensilla, if present, could not be identified.

Walking legs: (Fig. 1). Covered with abundant small- to medium-sized bifurcated setae, and a few longer setae. Legs II and III: tibia and basitarsus with array of pro- and retroventral rows of spiniform setae; on basitarsus apparently a row of three proventral, row of three retroventral, and one distal subventral spiniform setae, in a 2.2.3 pattern; telotarsus bi-segmented with pro- and retroventral rows of spiniform setae, each apparently with five and three, respectively, in a 1.1.2/2.2 pattern. Leg IV: Tibia with row of three/four spiniform setae on proventral surface and single distal spiniform seta on retroventral surface; basitarsus apparently with row of four provental and one distal retroventral spiniform setae, in a 1.1.1.2 pattern; telotarsus bi-segmented with incomplete (ventral) segmentation on first (basal) tarsomere, with pro- and retroventral rows of six spiniform setae each, in a 2.2.2-2/2.2 pattern.

Opisthosoma: (Figs. 1, 20–23). Tergites with abundant bifurcated setae of variable size. Sternites with several bifurcated setae. Ctenidia present on 3rd and 4th post-genital sternites (post-spiracular sternites I and II); ctenidia filiform and setae-like, similar in thickness to the bifid setae, but distinguishable because ctenidia are longer, single-tipped (non-bifid), and flexible; ctenidia similar in the two sternites. Post-spiracular sternite II with row of rigid hairs along posterior margin. Two pairs of microsetae, of the same type reported by Iuri et al. (2014) and Botero-Trujillo (2014), present in the posterior half of the genital plate, 1st and 2nd post-genital sternites (spiracular sternites); one of these microsetae is also present on each side of post-spiracular sternite I (these could not be seen in other sternites due to dense setation).

Female.—Meristic data in Table 1. Figs. 3–5, 7–10. Similar to male but larger and more robust; propeltidium wider. Ctenidia present in the same sternites and similar to those of male. Chelicera without the sexual specializations of males. Fixed and movable fingers very sharp, with sharp teeth. Fixed finger dorsal surface more elevated than manus, evidently curved on lateral aspect and without dorsal crest; fixed finger highest elevation at level of mucron. Movable finger mucron aculeus-like, with teeth located in a noticeably basal position on the finger; mucron cylindrical; vestigial retrolateral carina present on basal third of finger (where granulose) and on the apex (i.e., retrolateral edge carina); gnathal edge carina



Figures 16–23.—*Vempironiella aguilari* gen. nov., sp. nov. SEM images. Male paratypes (MACN-Ar-35454). 16. Right pedipalp, prolateral aspect; 17. Ibid., detail of telotarsus; 18. Tip of clubbed seta on pedipalp telotarsus; 19. Leg IV malleoli; 20. Genital plate; 21. Pair of microsetae on genital plate; 22. Post-spiracular sternite I (arrows indicate some ctenidia); 23. Post-spiracular sternite II (arrows indicate some ctenidia). Scale bars: 0.5 mm (Fig. 16); 0.1 mm (Figs. 17, 19, 20, 22–23); 5 μm (Fig. 18); 10 μm (Fig. 21).

obsolete, not elevated and identified only by a sclerotized line along the mucron dorsal margin.

Variation.—Dental pattern formula: In females and juveniles: FD-(1-2)-FM-(1-2)-FP-(1RFA, 1RFP, 1RFSP); in males: FD-(1-2)-FM-(1)-FP-(1RFA, 1RFP, 1RFSP).

Number of teeth on the FSD secondary teeth category: Males: n (chelicerae) = 24; 21 with one, 3 with two FSD; females: n = 4; 4 with two FSD.

Number of teeth on the FSM secondary teeth category: Males: n (chelicerae) = 24; 24 with one FSM; females: n = 4; 2 with one, 2 with two FSM.

Notes.—Resulting from a year-round (1974–1975) ecological study of the arthropod fauna of the Tillandsial of Punta Hermosa, Aguilar (1977: 91) reported *V. aguilari* [as "*Mummucia variegata* (?)"] as the most abundant arachnid species. Aguilar (1977) did not mention if the specimens were to be deposited in a collection; however, he speeified that some arachnid samples from his study had been sent to Dr. M. E. Galiano, formerly at the MACN where the material herein referred was found. The information contained in the label with the specimens accurately indicates that these are from Aguilar's survey of the spring of 1974 (September to November). Aceording to Aguilar (1977: fig. 4), around 40 specimens of only that solifuge species were captured in that season, while other, about 190 specimens were captured during the rest of the year (mostly in summer). So far, only the specimens here referred are known to be deposited in a formal collection, the rest remain unlocated.

Even though *V. aguilari* appeared to be, back then, fairly abundant throughout the year, a two-day survey to the type locality condueted by the author in early March 2014, aimed at collecting additional material of this species, was unsuccessful. Whether the population density might have deereased, or which variables might be related to the species not having been found, cannot be determined at this time.

Habitat.—The coastal-desert area where *V. aguilari* was collected is characterized by the presence of the xerophyte *Tillandsia latifolia* (Bromeliaceae) (Aguilar 1977).

Table 1.—Meristic data for *Vempironiella aguilari* gen. nov., sp. nov. Measurements in millimeters for male and female. L = length; W = width; H = height. [1]Measured along medial axis, from the propeltidium anterior margin to the opisthosoma posterior margin. [2]Measured in dorsal view at widest point. [3]Measured in retrolateral view parallel to longitudinal axis of chelicera, from the fixed finger apex to anterolateral propeltidial lobe anterior margin. [4]Measured in retrolateral view, along vertical axis at widest part of manus. [5]Sum of individual segment lengths. [6]Measurement excludes claws. * Range for males (n = 15). ** Measurement unavailable (legs IV absent).

	Male holotype (MACN-Ar-35453)	*Female paratype (adult)* (MACN-Ar-35454)
Total body L:		
With chelicerae:	5.72 (holotype) [3.90 – 5.85] *	8.11
w/o chelicerae:[1]	4.66 (holotype) [3.19 – 4.79] *	5.72
Propeltidium:		
L:	0.90	1.57
W:[2]	1.00	2.10
Chelicera:		
L:[3]	1.23	2.83
W:[2]	0.43	0.97
H:[4]	0.37	0.97
Pedipalp total L:[5]	3.37	5.33
Femur L:	1.17	1.83
Tibia L:	1.00	1.67
Tibia W:[2]	0.23	0.42
Basitarsus + telotarsus L:	1.20	1.83
Leg I total L:[5]	2.70	4.11
Patella L:	0.90	1.17
Tibia L:	0.82	1.30
Basitarsus L:	0.58	0.97
Telotarsus L:	0.40	0.67
Leg IV total L (w/o claws):[5]	4.57	**
Patella L:	1.50	**
Tibia L:	1.37	**
Basitarsus L:	1.03	**
Telotarsus L: [6]	0.67	**

DISCUSSION

The cheliceral morphology of *Vempironiella aguilari* is challenging to interpret, especially that of the movable finger. Bird et al. (2015) consider in corollary 1 of their structural criterion of homology that secondary teeth are more likely to be absent than primary teeth. On the other hand, corollary 2 argues that teeth are more prone to be absent the more distal its position is on the finger (except within secondary teeth categories where the opposite can be true).

The chelicerae of *V. aguilari* bear only two teeth on the movable finger, the proximal of which is larger than the distal. In interpreting this dentition pattern in the light of the corollaries of Bird et al. (2015), it could be argued that it is the MSM tooth which is absent, the smallest tooth on the movable finger of this species corresponding to MM. Three things suggest, however, that this is not the case and that it is the MM tooth which is indeed absent. First, in all known mummuciid species, the MM tooth closes just slightly proximal to its serial homolog on the fixed finger, FM; therefore if MM is presumed to be present in *V. aguilari*, then its closure with respect to FM would deviate from that pattern considering that the two teeth would be well distant when the fingers are closed. In addition, the two teeth on the movable finger of *V. aguilari* are placed in a clearly basal position on the finger, while the finger mucron is very long. If compared with other species in the family, it is reasonable to consider that it was the absence of MM tooth, instead of the MSM, which makes the mucron of this species that long as compared to the whole finger length. The absence of MM tooth would also more easily explain why the teeth are placed in a basal instead of median position on the finger, the latter being more widely distributed across mummuciid taxa. Likewise, the anterior-most tooth on the movable finger of *V. aguilari* is considerably smaller than MP, as it most frequently happens with MSM and MP teeth, respectively, throughout the order (Bird et al. 2015). Although the former tooth is indeed much more developed compared to the MSM of other species in family Mummuciidae, it is similar in size to the secondary teeth of the fixed finger, and therefore the hypothesis that it is the MSM tooth remains feasible.

The frfd in the chelicerae of *V. aguilari* involves the absence of retrofondal teeth (including RFM). This diastema, which is present in adults of both sexes as well as in juveniles, is to our knowledge not shared with any other described solifuge. The frfd is unlikely to be homologous to the fondal notch of many male eremobatids, the later of which is situated immediately proximal to the FP tooth, whereas the frfd is proximal to RFA. The frfd is not either considered homologous to the medial notch of some other families (e.g., Solpugidae), since such diastema is situated between FM and FSM and does not involve the lack of teeth (Bird et al. 2015).

The shape of the chelicerae of solifuges has been proposed to be associated with dietary preferences and burrowing abilities (Van der Meijden et al. 2012; Bird et al. 2015). For instance, species with multidentate chelicerae are presumed to be especially successful at hunting small, fast-running prey, at the cost of lower force as compared to species with robust chelicerae (Bird et al. 2015). The chelicerae of *V. aguilari* are neither multidentate nor especially robust, and these might also be associated with feeding and burrowing adaptations. The long and delicate shape of the fingers of *V. aguilari* suggests that these solifuges are not especially adapted for burrowing or that they burrow in soft substrates (e.g., loose-sand removal instead of hard-substrate excavation). In contrast, it is possible that the long, sharp aculeus-like movable finger can serve as a "killing weapon" that easily penetrates soft-bodied animals or articular membranes. In addition, the large size of the MP tooth might grant these animals the ability to break small, hard-shelled preys (e.g., ants), or to prevent them from escaping, for instance, by crushing them or keeping them trapped against the frfd. These hypotheses, however, have not yet been confirmed with live animals and direct observations will be necessary.

The cheliceral morphology of *V. aguilari* is remarkable and very different from that of most species in the family. Interestingly, there is some resemblance between the chelicerae of this species and that of *M. mauryi* (see Xavier & Rocha 2001). In both, the chelicerae are slender with finger tips sharp,

the movable finger mucron is long and delicate, and the fixed finger highest elevation in female is at level of the mucron. These resemblances probably result from independent adaptations in two distant taxa. As for the two other Peruvian species, *M. exlineae* and *M. masculina*, neither is assignable to the new genus and their systematic position remains to be determined.

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The first New World species of the pseudoscorpion family Feaellidae (Pseudoscorpiones: Feaelloidea) from the Brazilian Atlantic Forest

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Abstract. The first American species of the pseudoscorpion family Feaellidae is named from specimens collected in the Atlantic Rainforest biome of southern Brazil. The lack of specialized setae on the movable chelal finger suggests that it belongs to a new genus and new species, which we name *Iporangella* gen. nov. and *Iporangella orchana* sp. nov., respectively. The only known population of *I. orchana* is located near Iporanga, São Paulo, and juveniles of an unidentified species are recorded from Ilha da Queimada Grande.

Keywords: New genus, new species, Brazil, Serra do Mar, *Feaella*, morphology

Members of the pseudoscorpion family Feaellidae can be instantly recognized by their raptorial pedipalps with opposing processes on the trochanter and femur, large teeth on the chela, including some facing prolaterally, and two, four or six tubercles on the anterior margin of the carapace (e.g., Ellingsen 1906; Chamberlin 1931; Beier 1932, 1955, 1966; Harvey 1992, 2013). Feaellidae is one of the smallest pseudoscorpion families with only a single recognized genus, *Feaella* Ellingsen, 1906, and 12 described Recent species from Africa, the Indian region, the Seychelles Islands and northwestern Australia (Harvey 2013), as well as a recently discovered fossil species from Eocene Baltic amber deposits (Henderickx & Boone 2014). A second genus has been found in southeast Asian caves which differs from *Feaella* in several ways including the morphology of the coxal region which is highly modified (Harvey unpublished data; M. Judson, in litt.). The genus *Feaella* is divided into three subgenera: *F. (Feaella)* for those species with six anterior carapaceal lobes, *F. (Tetrafeaella)* with four lobes, and *F. (Difeaella)* with two lobes (Beier 1955, 1966; Harvey 2013). An alternative generic classification was developed in an unpublished Ph.D. thesis by Mark Judson (1992) and will be published in his forthcoming review of the family.

The first record of an American feaellid was made by Andrade (2003) who reported specimens from the Serra do Mar ecoregion of the Atlantic Forest biome in southern Brazil. The Atlantic Forest region has been heavily cleared and is now highly fragmented, although the Serra do Mar ecoregion is the most intact of the Atlantic Forest (Ribeiro et al. 2009). The Serra do Mar is listed as Critical/Endangered by the World Wildlife Fund (http://www.worldwildlife.org/ecoregions/nt0160; accessed 7 January 2016).

The specimens reported by Andrade (2003) are the subject of the present study, and are found to differ from other feaellids in the lack of specialized setae on the movable chelal finger. This difference is sufficient to warrant the formation of a new genus to accommodate the new species. We also record a population of the same genus from a nearby island, Ilha da Queimada Grande. These specimens cannot be identified to species level due to the lack of adult specimens.

The discovery of members of the family Feaellidae raises the number of pseudoscorpion families recorded from South America to 20, with only six families – Pseudogarypidae, Hyidae, Neobisiidae, Parahyidae, Lareidae and Sternophoridae – absent or not yet discovered from the region (Harvey 2013).

METHODS

The specimens examined during this study are lodged in the Museu de Zoologia da Universidade de São Paulo (MZSP), and the Instituto Butantan, São Paulo (IBSP). They were examined by preparing temporary slide mounts by immersing the specimen in 75% lactic acid at room temperature for one to several days, and mounting them on microscope slides with 10 or 12 mm coverslips supported by small sections of nylon fishing line. Specimens were examined with a Leiea MZ16 dissecting microscope, a Leica DM2500 or Olympus BH–2 compound microscope, and illustrated with the aid of a drawing tube. Measurements were taken in mm at the highest possible magnification using an ocular graticule. After study the specimens were rinsed in water and returned to 75% ethanol with the dissected portions placed in 12 × 3 mm glass genitalia microvials (BioQuip Products, Inc.). Some specimens (which have since been lost) were examined using a ZEISS DSM 940 scanning electron microscope located in the "Laboratório de Microscopia Eletrônica da Universidade de São Paulo".

Terminology and mensuration largely follow Chamberlin (1931), with the exception of the nomenclature of the

pedipalps, legs and with some minor modifications to the terminology of the trichobothria (Harvey 1992), chelicera (Harvey & Edward 2007; Judson 2007) and faces of the appendages (Harvey et al. 2012).

SYSTEMATICS

Family Feaellidae Ellingsen, 1906
Genus *Iporangella* gen. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:C15233BC-C021-4798-948A-8A992C94712E

Type species.—*Iporangella orchama* sp. nov.

Diagnosis.—*Iporangella* differs from all other feaellids by the lack of specialized setae on the retrolateral face of the movable chelal finger.

Description.—*Adult:* most setae short, inconspicuous, slightly curved and acuminate.

Chelicera (Fig. 4D): hand with 5 large and several small setae; *is* and *ls* adjacent to each other; movable finger with 1 subdistal seta; with 2 dorsal and 1 ventral lyrifissures; rallum of 1 long, slender blade (Fig. 4E); lamina exterior absent; movable finger short.

Pedipalp (Figs. 2H, 4F): trochanter with prolateral conical protuberance, femur without prolateral process, chela tubular. Fixed chelal finger and hand with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 4G): *esb* and *est* situated midway on retrolateral face; *ib*, *isb* and *ist* situated basally in straight row; *eb* and *it* situated subdistally, very close to each other; *et* situated distally, much closer to diploid trichobothrium (*dt*) than to *it*; *dt* situated distally; *st* situated sub-basally; *t* slightly closer to *sb* than to *b*. Movable chelal finger without specialized setae. Venom apparatus absent. Chelal teeth large and diastemodentate.

Carapace (Figs. 1C, 2D, 2E, 4A): anterior margin with 2 broad lobes; with 2 pairs of eyes situated on tubercles away from anterior carapaceal margin; all eyes with tapetum; with posterior furrow; without postero-lateral processes.

Coxal region (Figs. 2I, 4B): median maxillary lyrifissure situated basally near clivus; posterior maxillary lyrifissure absent. Coxa I without depression, each with 1 small coxal spine, situated basally (Fig. 4C); coxa II without coxal spines.

Legs (Fig. 5A): patellae with shallow dorsal depression; femora III and IV shorter than patellae III and IV; femora III and IV not solidly fused with patellae III and IV, respectively; metatarsi and tarsi fused; subterminal tarsal setae acuminate; sub-ungual spine present; arolium slightly shorter than claws.

Abdomen (Figs. 1A, 1B, 1D, 2A–C): very broad, nearly circular; tergite XI and sternite XI fused (Fig. 3F); tergite XII and sternite XII (anal sclerites) strongly sclerotized; tergite XII with 2 setae; anal region with raised circular rim. Sternite II of female absent (Fig. 5C); sternite III of male and female slender (Figs. 5B, 5C). Pleural membrane with numerous sclerotized pleural platelets in two rows (Figs. 1D, 3G), most platelets with a single seta.

Genitalia: details not visible.

Tritonymph: Pedipalp: fixed chelal finger with 7 major trichobothria, plus diploid trichobothria (*dt*), movable chelal finger with 3 trichobothria (Fig. 4H); *isb* and *sb* absent; *esb* and *est* situated midway on retrolateral face; *ib* and *ist* situated basally; *eb* and *it* situated medially; *et* situated closer to diploid trichobothrium (*dt*) than to *it*; *dt* situated distally; *t* situated closer to *st* than to *b*; movable finger without specialized setae. Carapace: with 2 anterior lobes; with 2 pairs of eyes.

Protonymph: Pedipalp: Fixed chelal finger with 2 major trichobothria, *eb* and *ist*, plus a single trichobothrium (*dt*); movable chelal finger with 1 trichobothrium, *t* (Fig. 4I); movable finger without specialized setae. Carapaee: with 2 anterior lobes; with 2 pairs of eyes.

Remarks.—The new genus most closely resembles *Feaella (Difeaella) krugeri* Beier, 1966 from South Africa, the only species currently included in the subgenus *Difeaella*, due to the presence of two lobes on the anterior margin of the carapace, and the lack of a basal prolateral process on the pedipalpal femur (Beier 1966). *Iporangella orchama* differs from all other feaellids, including *F. (D.) krugeri*, by the lack of specialized setae on the retrolateral face of the movable chelal finger. Although the presence of these setae was not mentioned in the original description by Beier (1966), they were reported by Judson (1992). *Iporangella orchama* further differs from *F. (D.) krugeri* by the location of the trichobothria: *ist* is situated basal to *esb* in *F. krugeri* (Beier 1966, fig. 5), but is distal to *esb* in *Iporangella* (Fig. 4G).

Etymology.—The generic epithet is derived from the type locality Iporanga, which is a municipality in São Paulo. The name comes from the Brazilian Indian language Tupi and means 'beautiful river'. The generic name is feminine in gender.

Iporangella orchama sp. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:F0AB5DDE-31DD-4CC5-AB98-BB93ED21D327
Figs. 1–5

Material examined.—*Holotype male.* BRAZIL: *São Paulo:* Iporanga, Vale do Ribeira, 24°33′34″S, 48°40′02″W, 19 March 2002, Winkler extraction, "raízes e folhiço" (roots and leaf litter), R. Andrade (MZUSP 67821).

Paratypes: BRAZIL: *São Paulo:* 1 ♂, same data as holotype except 8 March 2002 (MZUSP 67822); 1 ♀, same data (MZUSP 67817); 1 tritonymph, same data (MZUSP 67818); 1 tritonymph, same data (MZUSP 67819); 1 protonymph, same data (MZUSP 67820).

Diagnosis.—As for genus.

Description (adults).—*Color:* all sclerotized portions deep red-brown (Figs. 1A–E). All sclerotized portions coarsely tuberculate and reticulate.

Setae: most setae short, inconspicuous, slightly curved and acuminate.

Cerotegument: most surfaces covered with conspicuous cerotegument.

Chelicera (Fig. 4D): hand with 5 large and several small setae; *is* and *ls* adjacent to each other; movable finger with 1 subdistal seta; galea very thick, without rami; hand coarsely tuberculate, except for finger and basal third; fingers without teeth; rallum with 1 long, thin blade (Fig. 4E); serrula exterior with ea. 18 blades; lamina exterior absent.

Pedipalp (Figs. 2H, 4F): troehanter with long, curved prolateral conical protuberance, 1.78–2.06 (♂), 1.90 (♀), femur very robust, without triangular process on prolateral corner, 2.02–2.05 (♂), 1.97 (♀), patella conical 2.91 (♂), 2.73 (♀), chela tubular, chela (with pedicel) 3.88–4.00 (♂), 3.75 (♀),

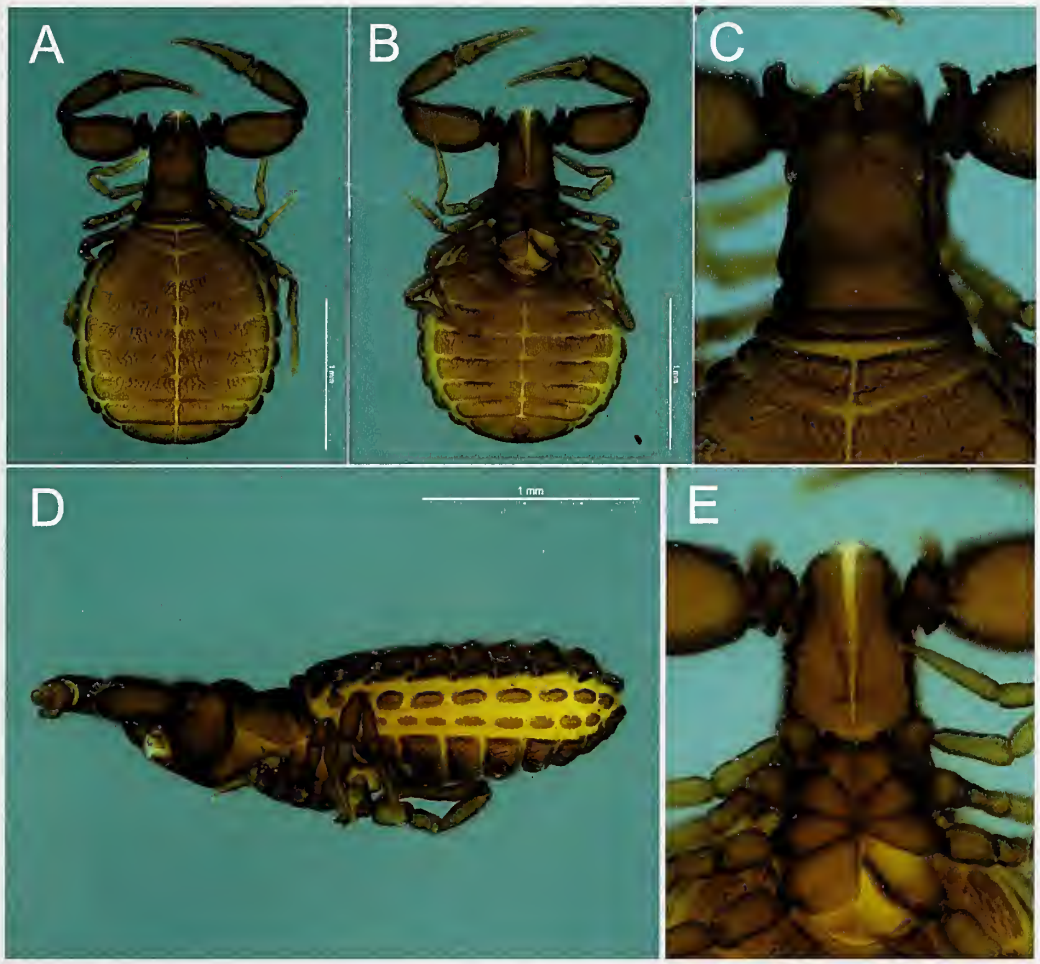

Figure 1.—*Iporangella orchama* sp. nov., holotype male (MZUSP 67821): A, body, dorsal view; B, body, ventral view; C, cephalothorax, dorsal view; D, body, lateral view; E, cephalothorax, ventral view.

chela (without pedicel) 3.58–3.73 (♂), 3.56 (♀), hand (without pedicel) 0.43–0.45 (♂), 0.42 (♀) x longer than broad, movable finger 5.80–6.31 (♂), 6.27 (♀) x longer than hand. Fixed chelal finger and hand with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 4G): *esb* and *est* situated midway on retrolateral face; *ib*, *isb* and *ist* situated subbasally in straight row; *eb* and *it* situated subdistally, very close to each other; *et* situated distally, much closer to diploid trichobothrium (*dt*) than to *it*; *dt* situated distally; *st* situated sub-basally; *t* slightly closer to *sb* than to *b*. Venom apparatus absent. Chelal hand very small; retrolateral condyle small and rounded; with dorsal protuberance at base of finger. Chelal teeth large, retrorse and diastemodentate: fixed finger with 12 (♂), 13 (♀) retrorse marginal teeth, 12 (♂, ♀) prolateral, 16 (♂), 18 (♀) retrolateral teeth, and 6–7 distal teeth; movable finger with 10 (♂, ♀) curved, marginal teeth and 12 (♂, ♀) prolateral teeth. Movable chelal finger without specialized setae.

Carapace (Fig. 1C, 2D, 2E, 4A): anterior margin with 2 broad lobes and deep antero-median depression; with 2 dorsal lobes on level with eyes; lateral margins nearly parallel; broadest basally; 1.26–1.27 (♂), 1.25 (♀) x longer than broad; with 2 pairs of well-developed eyes situated on tubereles away from anterior carapaceal margin; all eyes with tapetum; with numerous inconspicuous setae; posterior furrow present.

Coxal region (Figs. 1E, 2I, 4B): pedipalpal coxa: tuberculate lateral processes situated near pedipalpal foramen; with scattered small setae; manducatory process with 2 stout, long acuminate setae; median manducatory lyrifissure basal, situated near clivus; posterior manducatory lyrifissure absent. Coxa I without depression, and each with 1 small, anteriorly directed coxal spine situated basally (Fig. 4C); coxa II without coxal spines; coxa III meeting in mid-line.

Legs (Fig. 5A): patellae I and II slightly shorter than femora I and II; femora III and IV shorter than patellae III and IV; femora III and IV not solidly fused with patellae III and IV, respectively; all patellae with shallow dorsal depression; metatarsi and tarsi fused; tarsi long and slender, without tactile seta; subterminal tarsal setae acuminate; sub-ungual spine present; claws smooth (Figs. 2K, 2L); arolium about same length as claws, with fimbriate distal margin (Figs. 2K, 2L).

Abdomen (Figs. 1A, 1B, 1D, 2A–C): broadly ovate; tergites II–X and sternites IV–X with distinct median suture lines; most tergites gently curved; tergite XI and sternite XI fused (Fig. 3F); tergite XII and sternite XII (anal sclerites) strongly sclerotized; most segments with several setae, generally arranged in a single irregular row along posterior margin of sclerite; tergite XII and sternite XII each with 2 setae; anal region with raised circular rim. Tergites and sternites with

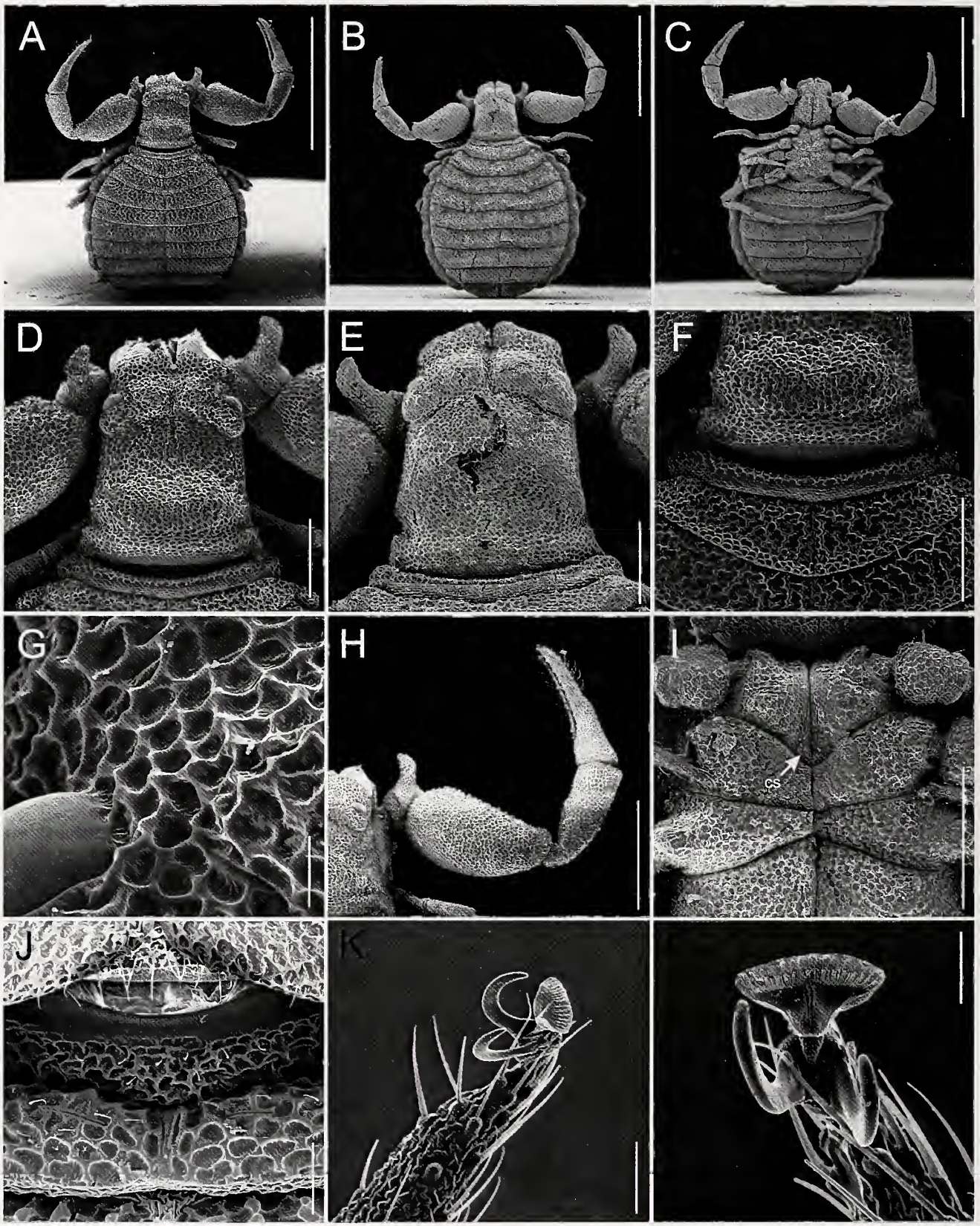

Figure 2.—*Iporangella orchama* sp. nov., scanning electron micrographs of adult specimens: A, body, dorsal view, specimen 1; B, body, dorsal view, specimen 2; C, body, ventral view, specimen 2; D, cephalothorax, dorsal view, specimen 1; E, cephalothorax, dorsal view, specimen 2; F, junction between cephalothorax and abdomen, dorsal view, specimen 1; G, detail of carapace near left anterior eye, specimen 1; H, right pedipalp, dorsal view, specimen 1; I, cephalothorax, ventral view, specimen 1; J, genital region, ventral view, male; K–L, tarsus, claws and arolium, unknown specimen. Scale lines = 1 mm (A–C); 0.4 mm (H); 0.2 mm (D–F, I); 0.04 mm (J); 0.02 mm (G, K); 0.01 mm (L).

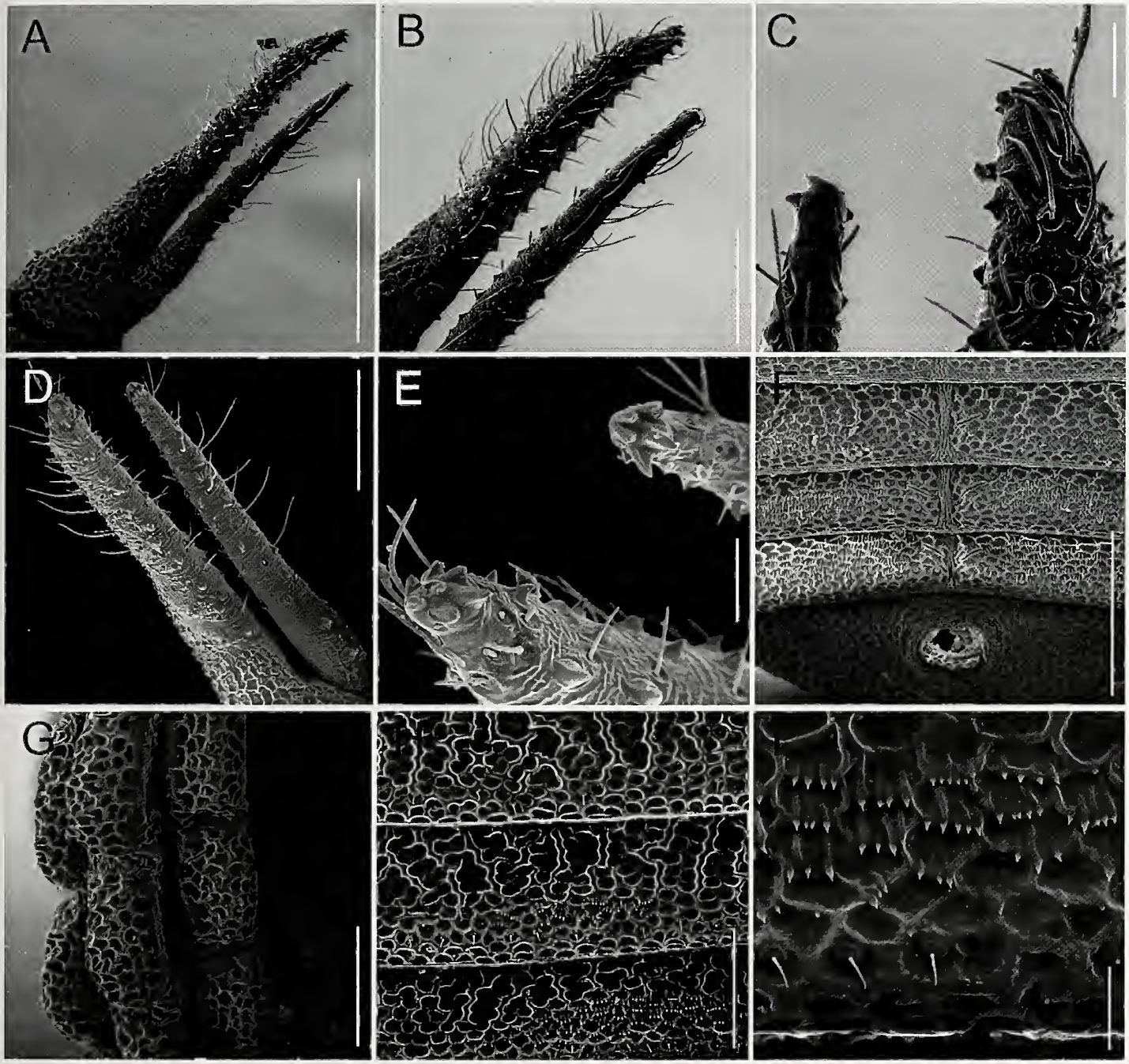

Figure 3.—*Iporangella orchama* sp. nov., scanning electron micrographs of adult specimens: A, right chela, retrolateral view; B, right chelal fingers, retrolateral view; C, left chela, tip of fingers; D, left chela, prolateral view; E, left chela, tip of fingers; F, abdominal sternites VII–XII, ventral view; G, pleural membrane; H–I, tergite, dorsal view. Scale lines = 0.2 mm (A, F); 0.1 mm (B, D, G, H); 0.02 mm (C, E, I).

posteriorly-directed micro-spinules (Fig. 3F, 3H, 3I). Most setae very small and inconspicuous; setae of male sternite II longer (Fig. 5B). Pleural membrane with 2 lateral rows each composed of 9 platelets, the dorsal ones larger (Fig. 1D); platelets with setae. First pair of spiracles connected to anterior margin of sternite IV, spiracular opening round and conspicuous; second pair of spiracles connected to anterior margin of sternite V, spiracular opening slit-like. Sternite II of male small and poorly sclerotized (Fig. 5B), of female absent (Fig. 5C). Sternite III of male (Fig. 5B) and female (Fig. 5C) slender and anteriorly curved.

Genitalia: details not visible.

Dimensions (mm): Males: holotype, followed by other male (when measured): Body length 2.24 (2.15); abdomen width (without pleura) 1.25 (1.15). Pedipalp: trochanter 0.32/0.18 (0.32/0.155), femur 0.635/0.315 (0.625/0.305), patella 0.495/0.17 (0.48/0.165), chela (with pedicel) 0.64/0.165 (0.60/0.15), chela (without pedicel) 0.59 (0.56), hand (without pedicel) length 0.075 (0.065), movable finger length 0.435 (0.41). Chelicera 0.235/0.14; movable finger 0.085. Carapace 0.625/0.495 (0.615/0.485); anterior eye diameter 0.055, posterior eye diameter 0.06. Leg I: femur 0.30/0.08, patella 0.23/0.085, tibia

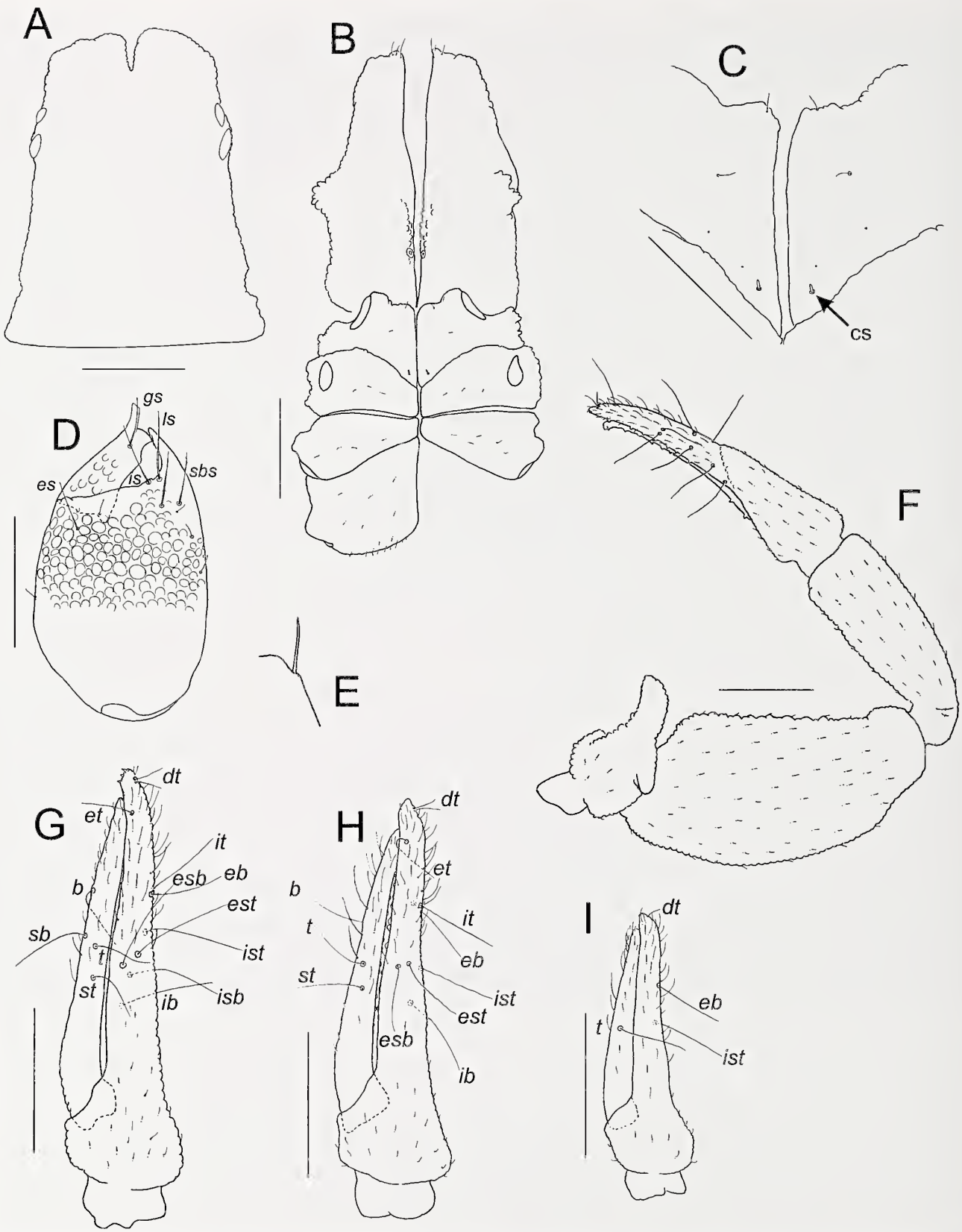

Figure 4.—*Iporangella orchama*, sp. nov., male holotype (MZUSP 67821), unless stated otherwise: A, carapace, dorsal view; B, cephalothorax, ventral view; C, coxae I, ventral view (cs = coxal spine); D, left chelicera, dorsal view; E, left rallum; F, right pedipalp, dorsal view; G, left chela, lateral view; H, left chela, lateral view, tritonymph paratype (MZUSP 67818); I, left chela, lateral view, protonymph paratype (MZUSP 67820). Scale lines = 0.2 mm (A, B, F–I); 0.1 mm (C, D).

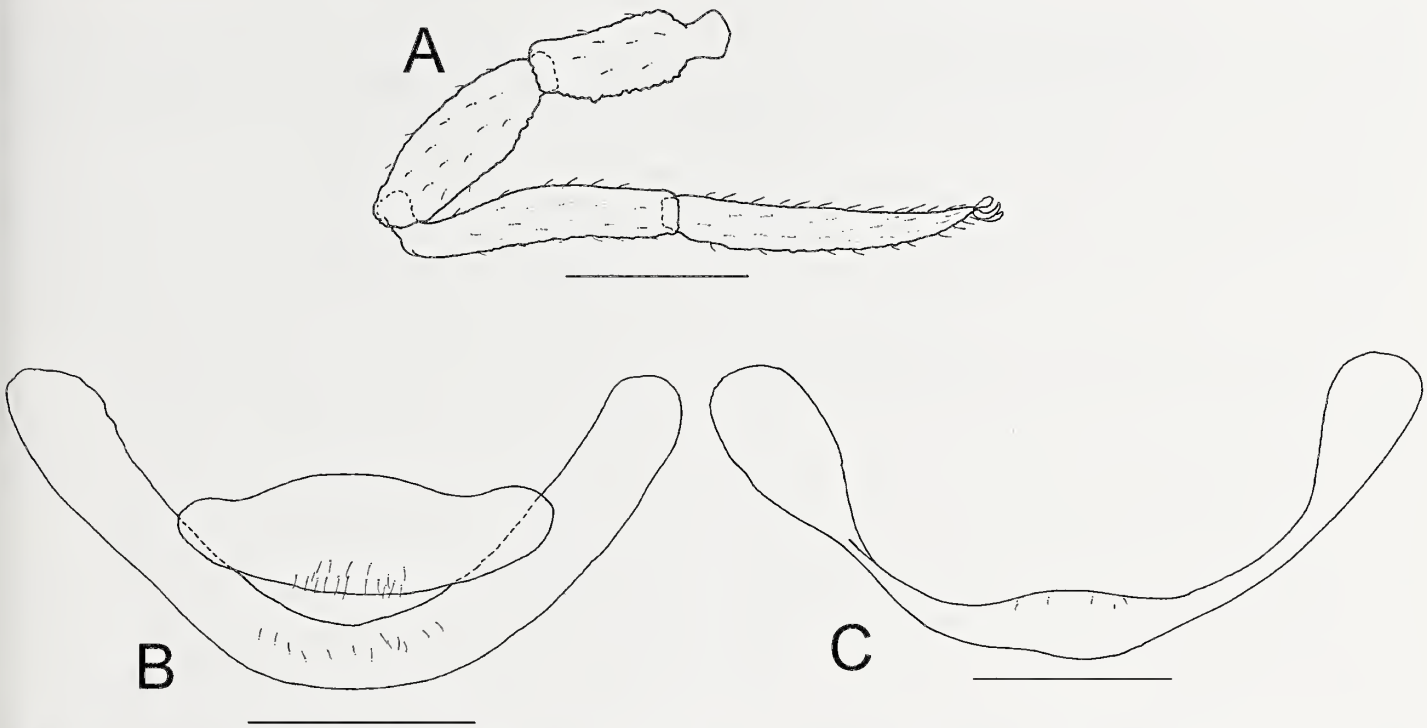

Figure 5.—*Iporangella orchama* sp. nov., male holotype (MZUSP 67821), unless stated otherwise: A, left leg IV, lateral view; B, genital sclerites (sternites II and III), ventral view; B, genital sclerite (sternite III), ventral view, female paratype (MZUSP 67817). Scale lines = 0.25 mm (A); 0.2 mm (B, C).

0.21/0.065, tarsus 0.33/0.055. Leg IV: femur 0.28/0.10, patella 0.32/0.115, tibia 0.425/0/075, tarsus 0.425/0.06.

Females: paratype: Body length 2.38; abdomen width (without pleura) 1.42. Pedipalp: trochanter 0.38/0.20, femur 0.74/0.375, patella 0.52/0.19, chela (with pedicel) 0.675/0.18, chela (without pedicel) 0.64, hand (without pedicel) length 0.075, movable finger length 0.47. Chelicera 0.25/0.155; movable finger 0.09. Carapace 0.675/0.54; anterior eye diameter 0.055, posterior eye diameter 0.05. Leg I: femur 0.31/0.09, patella 0.25/0.09, tibia 0.23/0.07, tarsus 0.35/0.06. Leg IV: femur 0.325/0.10, patella 0.35/0.12, tibia 0.45/0.075, tarsus 0.435/0.095.

Description (tritonymph).—*Chelicera:* movable finger with 1 seta.

Pedipalp: femur 2.04, patella 2.75, chela (with pedicel) 4.21, chela (without pedicel) 3.89, hand 0.68 x longer than broad. Fixed chelal finger with 7 major trichobothria, plus diploid trichobothria (*dt*), movable chelal finger with 3 trichobothria (Fig. 4H); *isb* and *sb* absent; *esb* and *est* situated midway on retrolateral face; *ib* and *ist* situated basally; *eb* and *it* situated medially; *et* situated subdistally; *dt* situated distally; *t* situated closer to *st* than to *b*; movable finger without specialized setae.

Carapace: 1.23 x longer than broad; with 2 anterior lobes; with 2 pairs of eyes.

Coxal region: coxa I without depression, and each with 1 small coxal spine situated basally.

Legs: much as in adult.

Dimensions (mm): Body length 2.28. Pedipalp: femur 0.57/0.28, patella 0.11/0.16, chela (with pedicel) 0.59/0.14, chela (without pedicel) 0.545, hand length 0.095, movable finger length 0.39. Carapace 0.57/0.465.

Description (protonymph).—*Chelicera:* movable finger without seta.

Pedipalp: femur 2.06, patella 2.77, chela (with pedicel) 3.67, chela (without pedicel) 3.43, hand 0.62 x longer than broad. Fixed chelal finger with 2 major trichobothria, *eb* and *ist*, plus a single trichobothrium (*dt*); movable chelal finger with 1 trichobothrium, *t* (Fig. 4I); movable finger without specialized setae.

Carapace: 1.06 x longer than broad; with 2 anterior lobes; with 2 pairs of eyes.

Coxal region: coxa I without depression, and each with 1 small coxal spine situated basally.

Legs: much as in adult.

Dimensions (mm): Body length 1.25. Pedipalp: femur 0.35/0.17, patella 0.36/0.13, chela (with pedicel) 0.385/0.105, chela (without pedicel) 0.36, hand length 0.065, movable finger length 0.265. Carapace 0.365/0.345.

Remarks.—The type series of *Iporangella orchama* consists of two adult males, a female and three nymphs. All were collected using Winkler extraction methods from leaf litter among the tangled thin roots of a tree. The site is situated near the lower slope of a mountain within the Serra do Mar ecoregion within the Atlantic Forest biome of southern Brazil. The Atlantic coastal forest has been heavily cleared since European settlement with only 11–16% of the original forest remaining (Ribeiro et al. 2009). The remaining forest is highly fragmented and only 9% is situated in protected areas (Ribeiro et al. 2009). The Serra do Mar ecoregion has been less heavily

cleared, probably due to its hilly topography, with an estimated 36.5% of the original forest remaining.

The scanning electron micrographs were obtained from specimens that have since been lost, and which are not regarded as part of the type series.

Etymology.—The specific epithet refers to this species being the first described from the New World (*orchamos*, Greek, first) (Brown 1956).

Iporangella sp. indet.

Material examined.—BRAZIL: *São Paulo*: 2 protonymphs, Ilha da Queimada Grande, Itanhaém Municipality, 24°29′S, 46°41′W, 30 April 2003, in pitfall traps, R. Indicati, C. Souza (IBSP 1620, 1623).

Description (protonymph).—*Chelicera:* movable finger without seta.

Pedipalp: femur 1.85, patella 2.09, chela (with pedicel) 3.80, chela (without pedicel) 3.50, hand 0.75 x longer than broad. Fixed chelal finger with 2 major trichobothria, *eb* and *ist*, plus a single trichobothrium (*dt*); movable chelal finger with 1 trichobothrium, *t*; movable finger without specialized setae.

Carapace: 1.20 x longer than broad; with 2 anterior lobes; with 2 pairs of eyes.

Coxal region: coxa I without depression, and each with 1 small coxal spine situated basally.

Legs: much as in adult.

Dimensions (mm): Body length 1.11. Pedipalp: femur 0.305/0.165, patella 0.23/0.165, chela (with pedicel) 0.38/0.10, chela (without pedicel) 0.35, hand length 0.075, movable finger length 0.255. Carapace 0.365/0.305.

Remarks.—Although these two protonymphs were collected only 125 km from the type locality of *I. orchama*, it is not possible to eonfirm whether they are conspecific with *I. orchama* until adult specimens are collected from Ilha da Queimada Grande.

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Microhabitat use and behavior differ across sex-age classes in the scorpion *Brachistosternus ferrugineus* (Scorpiones: Bothriuridae)

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Abstract. Intra- and interspecific coexistence has been recorded in several species of scorpions, reflecting different levels of aggregation and sociability. Some species of scorpions avoid temporal or spatial overlap of their surface activities, which may differ depending on species, age group or gender, and thus reduce intra- and interspecific competition and predation. We examined the surface activity of males, females and juveniles (sex-age class) of the scorpion *Brachistosternus ferrugineus* (Thorell, 1876) in an area of Arid Chaco, and also its microhabitat preference and behavior by each sex-age class. The month-by-month activity of each sex-age class was different, but all the classes were observed each month. The most frequently used microhabitat was *soil* (64.8%), while *leaf litter* and *vegetation* were used in similar proportions. The behavior most frequently observed was *ambush* (68.3%), followed by *walking* and less frequently *feeding*, *doorkeeping* and *courting*. Each sex-age class performed one particular behavior with more frequency than the others. Analyzing combinations of microhabitat, behavior and sex-age class, we found the juveniles were associated with *feeding* on *vegetation*, males with *walking* on *leaf litter*, while females were related to *ambush* on *soil*. No marked temporal distribution between sex-age classes was observed. However, the spatial distribution and frequency of behaviors were highly dependent on developmental stage and sex. These differences may facilitate understanding of the coexistence of different age-sex classes of *B. ferrugineus*.

Keywords: Arachnids, intraspecific coexistence, surface activity, Arid Chaco

Scorpions are primarily solitary and sedentary arthropods. They are excellent predators, preying on a wide variety of populations of insects, spiders and even other scorpions, and in turn are prey, mostly of vertebrates (Williams 1987; McCormick & Polis 1990; Polis 1990; Brownell & Polis 2001). They occur in a variety of terrestrial habitats and may be divided into two general groups based on microhabitat preference: ground-dwelling species, which live in burrows or under surface debris such as rocks or logs, and arboreal species, found at various heights on the vegetation (Polis 1990; Brown & O'Connell 2000). In deserts and semiarid regions, where scorpions are common, the majority of species are ground-dwellers (Polis 1990; Polis & Yamashita 1991). During the day, these species are relatively inactive and remain in their shelter or burrow. Individuals emerge from these diurnal retreats shortly after sunset to forage or engage in other activities, usually remaining on the ground surface near the burrow or shelter (Polis 1979; Polis et al. 1985).

Intra- and interspecific coexistence has been recorded in several species of scorpions (McReynolds 2004, 2008; Kaltsas et al. 2009; Shehab et al. 2011; Lira and De Souza 2014), reflecting different levels of aggregation and sociability (Polis & Lourenço 1986; Polis 1990). Some species of scorpions avoid temporal or spatial overlap of their surface activities, which may differ depending on species, age group, gender or body size. This is probably due to the presence of conspecifics and heterospecifics in the environment, which would result in substantial competition for food and shelter resources and may decisively influence habitat selection (Polis 1980, 1984; Due & Polis 1985; Polis & McCormick 1987; McReynolds 2008; Kaltsas et al. 2009; Lira et al. 2013). Habitat selection by an intermediate predator often means a balance between success in foraging and risk of predation (Murdoch & Sih 1978; Luttbeg & Schmitz 2000). Foraging success in scorpions may be associated with seasonal changes in prey availability (Polis 1980; Polis & McCormick 1986) and in the risk of predation by nocturnal predators, which may also be associated with the lunar cycle (Hadley & Williams 1968; Polis 1980; Polis et al. 1981; Nime et al. 2013). Thus, to increase predation success and to avoid being preyed upon, scorpions may modify their nocturnal activity or microhabitat use on nights with high illumination (Skutelsky 1996). For example, they may feed on trees or shrubs with high prey availability which, in turn, could provide protection against predators (McReynolds 2004).

The behavior and the substrate used by many species of scorpions are related, among other factors, with the sex-age class (Skutelsky 1996; Brown & O'Connell 2000; McReynolds 2004; Yamashita 2004). For example, walking behavior in several scorpion species is more associated with males than with females and juveniles (Polis & Farley 1979; Polis 1980; Yamashita 2004). Major displacement has even been observed in the males of many species, especially in the breeding season, as they actively search for females to mate with (Polis & Farley 1979; Polis & Sissom 1990; Araújo et al. 2010). Males of *Smeringurus mesaensis* (Stahnke, 1957) can travel more than 100 m in a night (with an average of 34.7 m), meanwhile, the females were observed up to one meter from their burrows (Polis & Farley 1979). Likewise, the behavior of climbing vegetation has been observed more frequently in juveniles than

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in adults of several species of scorpions (Bradley 1988; Polis 1990; Skutelsky 1996).

No previous studies have examined habitat use by *Brachistosternus ferrugineus* (Thorell, 1876) or its behavior in the months of activity. Only a few studies have addressed the ecology of scorpions in Arid Chaco regions (Acosta 1995; Nime et al. 2013, 2014). This lack is particularly surprising in view of the high biodiversity of such environments and the evidence that environmental change is transforming the ecology of arid regions, which are often predicted to be among the ecosystems most responsive to global climatic change (Hamerlynck et al. 2000; Whitford 2002).

The aims of this study were to analyze whether the surface activity of males, females and juveniles (sex-age class) of the scorpion *B. ferrugineus* varies from month to month in an area of Arid Chaco, and to examine whether each sex-age class has a preferred microhabitat and behavior. Our hypothesis is that sex-age classes of *B. ferrugineus* use different microhabitats and shows temporal displacement. The findings of this study on the ecology and behavior of this widespread and abundant species (Acosta 1995; Ojanguren-Affilastro 2005; Nime et al. 2013) are therefore important for understanding the processes of intra-specific coexistence and can contribute to a greater understanding of the structure of arthropod communities in the Argentine Arid Chaco.

METHODS

Study site.—The study was conducted in the Parque Provincial y Reserva Forestal Chancaní (Chancaní Reserve, 31°22′13.21″ S 65°27′13.75″ W, 4,960 ha). The reserve is located in the southernmost portion of the Chaco (Arid Chaco ecoregion, NT0701 in Olson et al. 2001) in Córdoba province, Argentina. Vegetation in the reserve is dry xerophilous woodland. The canopy is discontinuous and ~15 m high, dominated by *Aspidosperma quebracho-blanco* and *Prosopis flexuosa* trees. The shrub stratum (~4 m high) is thorny, dense, and almost continuous (Carranza et al. 1992; Cabido & Pacha 2002). The reserve supports forest stands that are close to climax conditions. The climate is highly seasonal, with a pronounced dry season. Annual rainfall averages 450 mm, concentrated during the summer (October–March). In the dry winter season (April–September), the water balance is negative, resulting in a soil humidity deficit. Mean annual temperature is 18 °C, with a mean value of 25 °C in the warmest month (January, with maximum temperatures reaching 45 °C during the day) and 10 °C in the coldest month (July) (Cabido & Pacha 2002).

There are four different ecological-type sites within the study site. (1) The *mature forest* site shows forest formations that are close to climax conditions, mainly with trees such as *A. quebracho-blanco* and *P. flexuosa*. The shrub layer is dominated by *Larrea divaricata*, *Mymozygantlus carinatus* and *Acacia furcatispina* (Carranza et al. 1992). (2) In December 1994, a high-intensity wildfire affected about 32,000 ha of Chaco forest, 230 ha of which are within the western boundaries of the Chancaní Reserve. Alongside the mature forest area, the fire generated a *secondary forest* area in which the vegetation is dense and homogeneous, dominated by high grasses (about 1 m tall) and shrubs about 2.5 m in height. In this area, young trees are common and dead trees are still standing (Pelegrin & Bueher 2010). (3) The *Jarillal* site is located in the central area of the reserve. In the past, the area was used for the logging of large trees for fuel and charcoal. Currently, it is dominated by shrubs of the *Larrea* genus ("jarilla"); the area is not used for any activities and is recovering. (4) The *forest with livestock* is a private area facing the reserve that has been used for years for raising livestock, so the site is much degraded with low, scattered shrubs.

Study animal.—The genus *Brachistosternus* Pocock, 1893 is second in richness within the family Bothriuridae Simon, 1880, containing 43 species described so far (Rein 2015; Ojanguren-Affilastro et al. 2016). The species of this genus inhabit arid and semi-arid environments from northern Ecuador to southern Patagonia in Argentina. Within Argentina, *Brachistosternus* species are dominant in xeric environments of the west and south, where they form large populations and are generally more abundant than other sympatric species (Acosta 1995; Ojanguren-Affilastro 2005; Nime et al. 2013; Ojanguren-Affilastro et al. 2016). They are ground-dwelling, living in burrows or under surface debris such as rocks or logs (Ojanguren-Affilastro 2005). Most species of *Brachistosternus* are active during the summer (from November to April), with generally a more extended activity period than other bothriurid genera (Ojanguren-Affilastro 2005). *Brachistosternus ferrugineus* are small to medium size scorpions (males between 27 to 43 mm, females between 35 to 56 mm) (Ojanguren-Affilastro 2005). Most of the localities where *B. ferrugineus* have been collected in Argentina are within the Chaco phytogeographic province, where it is the most common species (Acosta 1995; Ojanguren-Affilastro 2005). This species lives at low or medium altitudes, from 100 to 1500 meters above sea level (Ojanguren-Affilastro 2005).

Experimental design.—Three sites were selected within the Chancaní Reserve representative of "mature forest" (Fig. 1a), "secondary forest" (Fig. 1b) and "Jarillal" (Fig. 1d). An additional site, "forest with livestock", was added adjacent to the reserve (Fig. 1c) in order to cover the landscape's heterogeneity.

At each site, 15 transects (50 × 6 m) were established, separated from each other by 70 m. Transects, one after the other, passed along and on the main roads in each sector, due to the difficulty of accessing and observing scorpions inside the dense forest.

Sampling consisted of walking along each transect with a portable ultraviolet (UV) lamp. Irradiation with UV causes scorpions to fluoresce, which enables all stages of the active scorpion to be easily detected in the dark (Honetschlager 1965).

Sampling lasted about 2 h after dusk (from 21:00 to 23:00), as these were the main activity hours of scorpions at the study site. Sampling was conducted for three nights a month at each site, always close to the new moon phase, to avoid the effect of moonlight on scorpion activity (Nime et al. 2013). Sampling was conducted during the periods November 2009 through January 2010 and November 2010 through February 2011. The sampling effort was the same at each site on each recording night (2 hours/night), but the months in which each site was sampled were not the same (Table 1). February was sampled in only one year (2010) but sampling was carried out

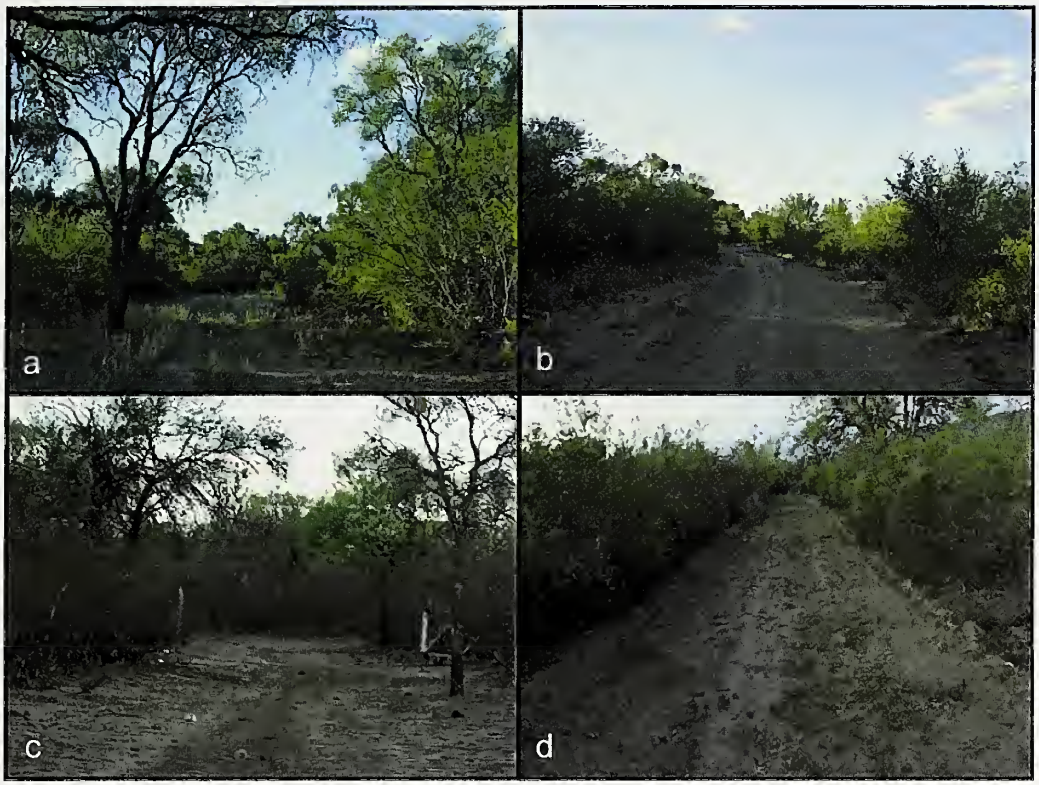

Figure 1.—Sampling sites within and outside the Chancaní reserve, Cordoba, Argentina. a. mature forest; b. secondary forest; c. forest with livestock; d. Jarillal.

in the other months in both seasons. Every month had the same number of sampling nights.

When a scorpion of *B. ferrugineus* species was located, it was observed and classified by sex-age class (male, female and juvenile). When necessary, scorpions were temporarily captured with forceps for identification. Every observation was made avoiding disturbances to other scorpions nearby. Specimens were not marked, since on a preliminary study the recovery of marked individual was too low (less than 2%). At the time of observation, we recorded the behavior of each scorpion and the microhabitat in which it was found. The behaviors reeorded were *ambush*, *feeding*, *walking*, *doorkeeping* and *courting*. *Ambush* was when the individual was at rest, with extended pedipalps (waiting for prey) or resting with the appendages in contact with the body. We attempted to record both behaviors (ambush and rest) separately according to the position of the pedipalps, but if they perceived our vibrations when we approached, they moved their pedipalps to the body, so we prefer not to discriminate between these two behaviors. The scorpion was classified as *feeding* when it was feeding on prey or when it had consumed the prey leaving only a small part between the chelicerae. The scorpion was classified as *walking* when it was observed moving. *Doorkeeping* behavior was when the animal was seen at the entrance of the burrow, either in ambush or entering. Finally, *courting* behavior is performed by scorpions prior to copulation (the male holding the female with their pedipalps), so only adults exhibit this behavior (Fig. 2).

The microhabitats considered were grouped into the following categories: *soil*, *leaf litter* and *vegetation*. *Soil* was considered when the individual was on bare ground, usually packed soil (Fig. 2). In *leaf litter*, the individual was on the leaf litter composed mainly of dry leaves (mostly from *A. quebracho-blanco* trees) and small branches. In *vegetation*, the individual was observed climbing vegetation, usually at the end or in the middle, not above 30 cm in height. Three scorpions were observed on cattle feces at the site with livestock, and this was considered as *leaf litter*.

We surveyed the availability of each microhabitat in the total area and in each site (with exception of jarillal). Three

Table 1.—Number of nights sampled for scorpions with UV light in the Chancaní reserve. * = no data.

Site	Nov.'09	Dec.'09	Jan.'10	Nov.'10	Dec.'10	Jan.'11	Feb.'11	Total
Mature forest	3	3	3	3	3	3	3	26
Secondary forest	3	3	3	3	3	3	3	26
Forest with livestock	*	3	3	3	3	3	3	18
Jarillal	*	*	*	3	3	*	*	6
Total	6	9	9	12	12	9	9	75

Figure 2.—*Brachistosternus ferrugineus* couple, male on the right and female on the left, *courting* on *soil*, in the secondary forest of Chancaní reserve, Córdoba, Argentina. Photo courtesy of María Eugenia Romero Lebrón.

quadrats (0.50 × 0.50 m) were placed to left, right and center of each transect (9 quadrats per transect) and the percentage (%) of *vegetation*, *leaf litter* and *soil* was recorded. The average of each microhabitat was then calculated at each site and for all of these.

Statistical analysis.—*Temporal distribution of the sex-age class:* Surface activity of scorpions (measured as the number of individuals active on the ground surface) of each sex-age class (males, females and juveniles) of the species *B. ferrugineus* and its variation between months were analyzed. Scorpion counts were modeled with a generalized linear mixed model (GLMM) using the glmer function of the lme4 library through the interface with R-packages (R Core Team 2013) implemented in InfoStat (Di Rienzo et al. 2013). We used a Poisson distribution (with a log-link function) because this is most appropriate for counts. The overdispersion was evaluated to ensure that the Poisson assumptions held. Sex-age class and month and their interaction were considered fixed effects. In order to take into account the repeated measures in months, site and transect were considered random effects. Significance level was set at 0.05. To compare means, we used the Fisher's least significant difference test (LSD).

Microhabitat preferences and behaviors associated with each sex-age class: First, contingency tables were used to evaluate if there were significant differences in proportions of microhabitat use by *B. ferrugineus* without discriminating by sex-age class. The same was done for the differences between proportions of behavior observed. Subsequently, a GLMM was performed to determine whether there were differences in proportions between classes within each microhabitat, using site, month and sex-age class as classification variables; sex-age class was selected as a fixed factor and site and month as random factors. The same analysis was performed for behavior.

Finally, a bi-plot obtained from multiple correspondence analyses (MCA) was performed to explore the associations between microhabitat, behavior and sex-age class. MCA takes multiple categorical variables and seeks to identify associations between levels of those variables. MCA allows exploring contingency tables by means of the decomposition of chi-square matrix. The contributions of each axis are indicated as a percentage of inertia (Abdi & Valentin 2007). The MCA results allow all the variables (behavior, microhabitat, sex-age class) to be observed together. Analyses were performed for the total sample and for each site separately to detect changes in associations due to the effects of site. Microhabitat, behavior and sex-age class were selected as classification criteria and frequency as the combination of these variables.

RESULTS

Temporal distribution of sex-age class.—We found a significant interaction between sex-age class and month for scorpion counts (Wald test, $\chi^2 = 25.60$, $P = 0.0003$). Due to the presence of the interaction, we performed a Fisher's LSD test for the combination of sex-age class and month. Females and juveniles had a higher surface activity than males in November. In January, the majority of active individuals that we saw were juveniles. In February, we observed more males and juveniles than females (Fig. 3). December showed no significant differences between the three classes. In general, the average number of active individuals of all three sex-age classes decreased from November through January, rising again in February (Fig. 3).

Microhabitat preference and behaviors associated with each sex-age class.—*Microhabitat availability: Soil* was the most abundant microhabitat in the study area (54.9%), while *leaf litter* (23.7%) and *vegetation* (21.3%) were similar in proportion. *Soil* was also the most abundant microhabitat in the three sampled sites (from 50.4 to 60.2%, see Table 2), followed by *leaf litter* in the mature forest site, and *vegetation* in the secondary forest. In the forest with livestock, there were similar percentages of both *vegetation* and *leaf litter* (Table 2).

Microhabitat preference: The results showed a significant difference between proportions of microhabitat used for total scorpions (Chi-square test, $\chi^2 = 702.32$, $P < 0.0001$). The most frequently used microhabitat for total scorpions was soil (64.8%), while leaf litter (18.1%) and vegetation (17.1%) were used in similar proportions, matching microhabitat availability. Table 3 shows the number of individuals of each sex-age class in different microhabitats at each site.

However, we detected significant differences between counts of sex-age classes in *leaf litter* and *vegetation* microhabitats (Table 4, Fig. 4). Males and females were significantly more abundant than juveniles in the *leaf litter* microhabitat. Juveniles were significantly more abundant in *vegetation*, followed by females and then the males (Table 4). No significant differences were found between the classes in the use of *soil* (Table 4).

Frequencies of behavior: The results showed a significant difference in proportions of observed behavior for total scorpions (Chi-square test, $\chi^2 = 3693.46$, $P < 0.0001$). *Ambush* behavior was the most frequently observed (68.3%), followed by *walking* (20.6%) and less frequently *feeding* (7.6%), *door-keeping* (2.7%) and *courting* (0.8%). Table 5 shows the number of individuals in each sex-age class performing different behaviors at each site.

In the analysis of sex-age classes and behaviors, significant differences were detected for some behaviors (Table 6, Fig. 5).

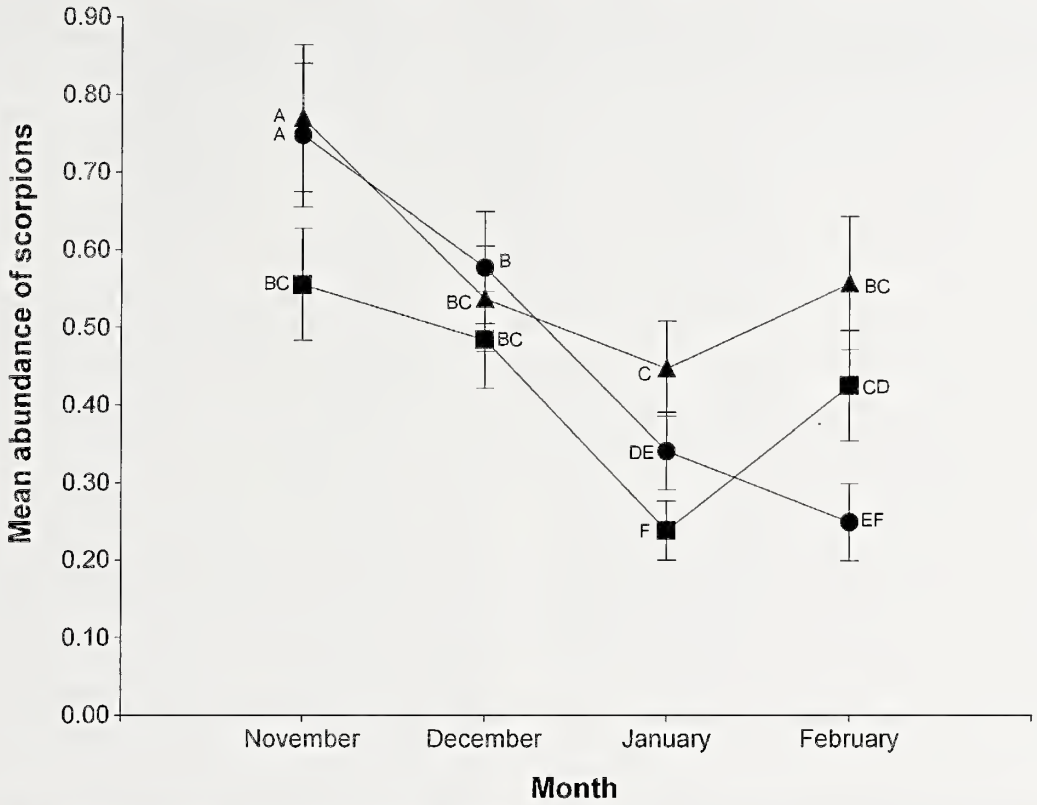

Figure 3.—Number of *Brachistosternus ferrugineus* scorpions, males (squares), females (circles) and juveniles (triangles), each month in the Chancaní reserve. Mean expressed as average number of scorpions by transect (± SE). Common letters are not statistically different (Fisher's LSD test, $p > 0.05$).

Ambush behavior was performed more frequently by females and juveniles than males. Juveniles were observed *feeding* more frequently than adults (males and females), and male adults did so less frequently than females. Females were observed more *doorkeeping* than males and juveniles. Most of the time, the females were found with the metasoma outside the burrow, as if entering the burrow (in 93% of observations). *Courtship* behavior was observed in six pairs of scorpions.

Combination of microhabitat, behavior and sex-age class.—Significant differences were observed between proportions of microhabitats used and sex-age class (Chi-square test, $\chi^2 = 777.38$, $P < 0.0001$), and between behavior and sex-age class (Chi-square test, $\chi^2 = 3188.31$, $P < 0.0001$). The bi-plot obtained by multiple correspondence analyses, performed for the total sample using sex-age class, behavior and microhabitat, reveals that juveniles were more likely to be *feeding* on *vegetation* (Fig. 6). While the majority of males were observed *walking* on the *leaf litter*, the females were related to *ambush* on *soil* (Fig. 6). Even when the four sites were analyzed separately, the pattern observed was very similar.

Table 2.—Percentage of microhabitat available (mean ± SD) in three sampled sites of the Chancaní reserve.

Site	Soil	Leaf litter	Vegetation
Mature forest	50.4 ± 16.1	30.6 ± 14.4	18.7 ± 13.5
Secondary forest	53.9 ± 14.3	20.0 ± 12.9	26.0 ± 13.9
Forest with livestock	60.2 ± 14.3	20.6 ± 11.5	19.2 ± 8.6

DISCUSSION

Temporal distribution of sex-age class.—The surface activity of males, females and juveniles was higher in November, and declined over the months in all three classes. However, all sex-age classes remained active during all the months sampled. The decrease was more marked in females; they did not have the increase in February that was observed in the other classes (Fig. 3). This may be because, after getting pregnant, the

Table 3.—Counts of *Brachistosternus ferrugineus* occurrence in each microhabitat within each site in Chancaní reserve, during the period of sampling.

Site	Sex-age class	Soil	Leaf litter	Vegetation	Total
Mature forest	Males	126	43	15	184
	Females	108	41	47	196
	Juveniles	104	43	83	230
Secondary forest	Males	93	24	5	122
	Females	125	38	19	182
	Juveniles	118	12	54	184
Forest with livestock	Males	42	16	2	60
	Females	64	27	7	98
	Juveniles	73	7	20	100
Jarillal	Males	53	12	0	65
	Females	30	9	3	42
	Juveniles	56	5	7	68
Total		992	277	262	1531

Table 4.—Generalized linear mixed models. Relationship between the surface activity of males, females and juveniles of *Brachistosternus ferrugineus* and each microhabitat used in the Chancaní reserve. Average monthly scorpions ± standard error of the mean. Means with a letter in common are not significantly different (Fisher's LSD, $P > 0.05$).

Microhabitat	χ^2	P	Males	Females	Juveniles
Soil	2.12	0.3463	40.99 ± 6.83 A	42.68 ± 7.10 A	45.82 ± 7.59 A
Leaf litter	12.93	**0.0016**	12.92 ± 2.08 A	15.64± 2.42 A	9.11 ± 1.58 B
Vegetation	124.89	**<0.0001**	2.93 ± 0.74 C	10.12 ± 1.82 B	21.83 ± 3.48 A

females stay sheltered in their burrows (Mahsberg 2001; Kaltsas et al. 2008).

The higher surface aetivity for the three classes in November could be because, in this region of the southern hemisphere, scorpions generally begin to leave their shelters or burrows and surface during spring when temperatures begin rising (Warburg & Polis 1990; Ojanguren-Affilastro 2005; Yamaguti & Pinto-da-Rocha 2006; Nime et al. 2013) to begin feeding after the winter and probably find a partner. We did not observe marked differences in temporal distribution between sex-age classes, as exists in many species of scorpions to avoid intra- and interspecific competition and predation (Polis 1980, 1984; Due & Polis 1985; Polis & McCormick 1987).

Microhabitat preference and behaviors associated with each sex-age class.—The microhabitat most used in all classes of *B. ferrugineus* was *soil,* and *ambush* was the most eommon behavior. When we examined all sex-age classes of scorpions together, we found that microhabitats were used in proportion to their availability. On the another hand, the most common behavior expected was *ambush*, because active looking for prey is not common in scorpions (McCormick & Polis 1990) since it involves a significant expenditure of energy (Kaltsas et al. 2008). Kaltsas et al. (2008) found that the most eommon behavior (over 84%) in looking for food for all sex-age classes of *Mesobuthus gibbosus* (Brulli, 1832) species was "sit-and-wait" (*ambush*), coineiding with our observations.

However, the correspondence analysis clearly demonstrated one eombination of a behavior and microhabitat was associated with each sex-age class. Male scorpions were mostly seen *walking* on *leaf litter*, females were more likely to be seen engaging in *ambush* behavior on *soil*, and juveniles were associated with *feeding* in *vegetation*.

That *walking* behavior is more associated with males was also observed in the species *Centruroides vittatus* (Say, 1821) (Polis 1980; Yamashita 2004). Males of this species are more active (54.4% walking on the soil surface) than females (34.9%). Also, males had a greater displacement, with marked individuals being found many meters away from the initial site, while females were found a few meters from the site, even after several weeks (Yamashita 2004). This increased movement of males is observed mainly in the breeding season as they actively search for females to mate (Polis & Farley 1979; Polis & Sissom 1990; Araújo et al. 2010).

In the present study, *doorkeeping* behavior was performed more often by females than by males and juveniles. Similar results were observed in females of *M. gibbosus*; these fed and looked for prey at the entrance of their burrows ("*doorkeeping*" strategy) more than males and juveniles, who were preferably near or far from their burrows (Kaltsas et al. 2008).

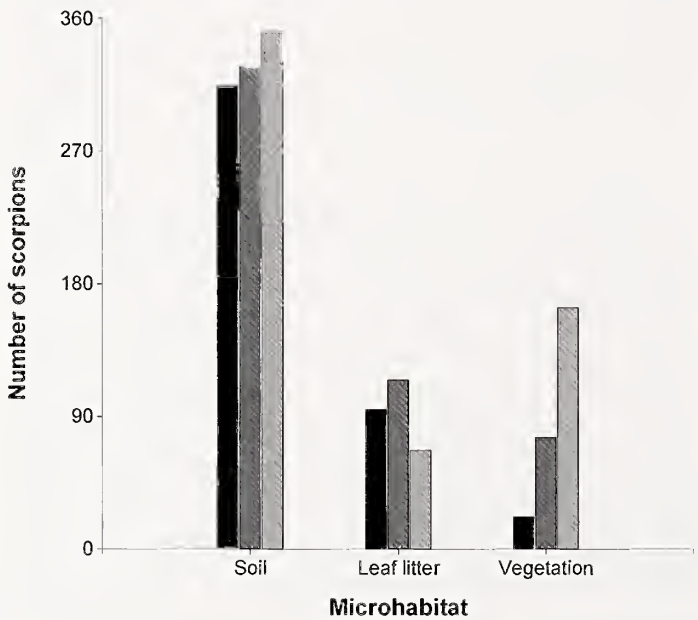

Figure 4.—Surface activity of males (black bars), females (dark grey bars) and juveniles (light grey bars) of *Brachistosternus ferrugineus* in different microhabitats in the Chancaní Reserve. Bars represent total number of scorpions observed.

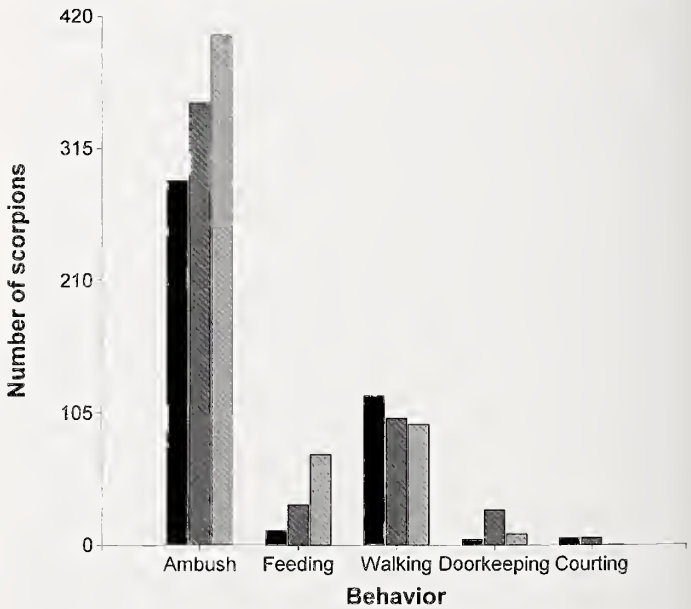

Figure 5.—Surface activity of males (black bars), females (dark grey bars) and juveniles (light grey bars) of *Brachistosternus ferrugineus* in the performing of different behaviors in the Chancaní Reserve. Bars represent total number of scorpions observed.

Table 5.—Counts of *Brachistosternus ferrugineus* performing each behavior in each study site of the Chancaní reserve.

Site	Sex-age class	Ambush	Feeding	Walking	Doorkeeping	Courting	Total
Mature forest	Males	114	9	57	1	3	184
	Females	144	11	31	7	3	196
	Juveniles	161	31	34	4	0	230
Secondary forest	Males	81	1	36	3	1	122
	Females	119	9	41	12	1	182
	Juveniles	125	22	36	1	0	184
Forest with livestock	Males	39	2	19	0	0	60
	Females	59	10	22	7	0	98
	Juveniles	65	15	17	3	0	100
Jarillal	Males	55	0	7	1	2	65
	Females	29	2	7	2	2	42
	Juveniles	54	4	9	1	0	68
Total		1045	116	316	42	12	1531

In the "*doorkeeping*" hunting strategy, the scorpion is located at the entrance to the burrow or refuge and waits for prey to approach (Benton 2001). Most females of *M. gibbosus* had recently mated. Therefore, staying close to their burrows is probably due to the maternal protective instinct (Mahsberg 2001; Kaltsas et al. 2008). Furthermore, foraging far from the burrow requires tolerance to adverse environmental conditions and inter- and intraspecific competition. In the open area, where males and juveniles of *M. gibbosus* feed mainly using the "*sit and wait*" strategy, temperature and relative humidity are comparatively lower, wind speed is higher and the moon is an important factor, while in the burrows of females, generally under the vegetation, environmental conditions are better (Kaltsas et al. 2008). Probably staying at the entrance is advantageous as there is a microclimate inside and environmental conditions are more favorable, and they have a shelter near to hide from predators. The females of *M. gibbosus* observed at the entrance of their burrows had only their pedipalps and sometimes their prosoma visible. In our study, the most common behavior of females of *B. ferrugineus* when doing *doorkeeping,* was at the entranee of their burrows with their metasoma outside, as if entering the burrow (in 93% of observations). The reasons why scorpions prefer a backwards position at the burrow entrance are unknown; they may have been entering in their burrows in flight after sensing the approach of collectors. Burrowing scorpions are very sensitive to vibrations in the ground (Warburg & Polis 1990).

We found the juveniles associated with *feeding* in *vegetation.* The behavior of climbing the vegetation has been previously observed in other species of scorpions (Williams 1970; Polis 1979; Bradley 1988; Cao 1993; Skutelsky 1996; Brown & O'Connell 2000; McReynolds 2004, 2008). In *Paruroctonus utahensis* (Williams, 1968), more juveniles than adults were observed in the vegetation (Bradley 1988). Juveniles of *Buthus occitanus* (Amoreux, 1789) were found in the bushes at a rate ten times higher than that of adults (Skutelsky 1996).

Although the reason for climbing behavior in the vegetation is unclear, there are some hypotheses such as decreasing the risk of predation, or increasing feeding success by foraging in an area with higher prey availability (Bradley 1988; Polis 1990; Brown & O'Connell 2000). The first hypothesis suggests that climbing is a behavior to avoid predation and assumes that the risk of predation is lower in vegetation than on the soil. Two observations are consistent with this hypothesis (Brown & O'Connell 2000). First, climbing has been seen generally in small species and juveniles of larger species (Polis 1979; Bradley 1988; Skutelsky 1996). In this study, *B. ferrugineus* is one of the smallest species in the area and the only one that we observed to climb vegetation. As these species or individuals probably have a wide range of predators that live on the soil (including larger intra- and interspecific scorpions; Polis & McCormick 1987), climbing could reduce meeting potential predators. Also, it was noted that *C. vittatus* moves on to vegetation during the phase of the moon with greater light intensity (50–100%) (McReynolds 2004). In open areas, scorpions are more visible to predators at night and so such a change in microhabitat use during the lunar cycle behavior may reduce the risk of predation when the illumination of the moon is high (McReynolds 2004). The present study could not test this, because sampling was always performed on moonless nights. Second, some species have been seen to take prey

Table 6.—Generalized linear mixed models. Relationship between the surface activity of males, females and juveniles of *Brachistosternus ferrugineus* in each behavior observed in the Chancaní reserve. Average monthly scorpions ± standard error. Means with a letter in common are not significantly different (Fisher's LSD, $P > 0.05$).

Behavior	χ^2	P	Males	Females	Juveniles
Ambush	19.51	**0.0001**	38.48 ± 5.94 B	46.74 ± 7.12 A	53.93 ± 8.15 A
Feeding	49.33	**<0.0001**	1.53 ± 0.53 C	4.09 ± 1.08 B	9.19 ± 2.11 A
Walking	2.73	0.2548	11.67 ± 5.19 A	9.90 ± 4.42 A	9.41 ± 4.21 A
Doorkeeping	19.19	**<0.0001**	0.60 ± 0.30 B	3.24 ± 1.00 A	1.08 ± 0.44 B
Courting	9.73	**0.0077**	0.32 ± 0.26 A	0.32 ± 0.26 A	0 ± 0 B

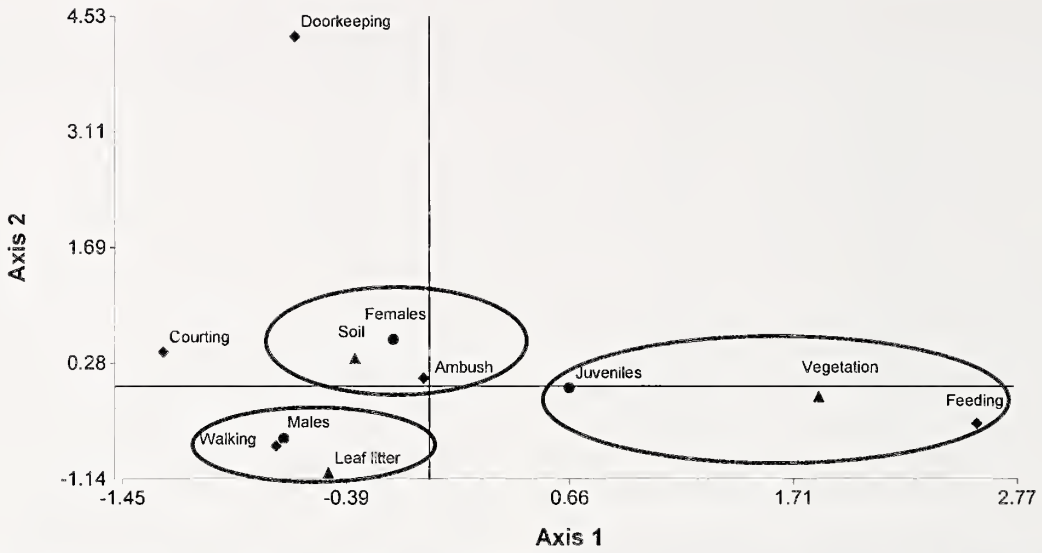

Figure 6.—Bi-plot obtained by multiple correspondence analysis. The principal axes represents the gradient in the relationship among the categorical variables sex-age class, behavior and microhabitat of *Brachistosternus ferrugineus*; categories close in the graph are more related.

captured on soil to vegetation before consumption, perhaps in order to reduce the chance of encountering a predator that might cause death or the loss of the prey while trying to escape (Polis 1979; Cao 1993; Brown & O'Connell 2000). In *Smeringurus mesaensis* this was age-specific behavior, with a significantly higher proportion of juveniles than of adults consuming prey in the vegetation (Polis 1979). In the present study, after the juveniles, females of *B. ferrugineus* were more frequently observed in the vegetation. The same occurs in *C. vittatus*, with females climbing more than males (Brown & O'Connell 2000; Yamashita 2004).

The second hypothesis suggests that prey abundance is higher in the vegetation, so feeding there would be more energy efficient (Bradley 1988; Polis 1990). However, although scorpions have been observed feeding while on vegetation, evidence of active foraging there is scarce (Polis 1990; Skutelsky 1996).

Possibly the vegetation, which creates a more complex environment than environmental deserts, offers juveniles more opportunities for hiding and reducing overlap with adults (Höfer et al. 1996). Habitat complexities reduce niehe overlaps and may reduce the need of temporary displacement (Yamashita 2004). Based on our results, we hypothesize that the behavior of climbing into vegetation is performed by juveniles for the purpose of feeding without risk of losing the prey and avoiding being preyed on in turn. However, this hypothesis requires testing.

Our hypothesis that sex-age classes of *B. ferrugineus* use different microhabitats while showing temporal displacement is rejected; sex-age classes did use different microhabitats, but without temporal displacement. We conclude that the microhabitat use and behavior frequencies were highly dependent on developmental stage and sex. The differences observed may facilitate the age-sex class coexistence of *B. ferrugineus* and may reduce the need of temporary displacement due to the risk of predation and competition for feeding. Also, this species is the only one that uses vegetation in the study area, and this possibility of using different niches could be one reason why *B. ferrugineus* is the most abundant and conspicuous species in the area, despite being physieally one of the smallest (Nime et al. 2013). The findings of this study on the ecology and behavior of this widespread speeies are therefore important for understanding the processes of intraspecific coexistence and can contribute to a greater understanding of the structure of arthropod communities in the Argentine Arid Chaco, and to general knowledge of scorpion ecology.

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SHORT COMMUNICATION

Leucism in *Tityus pusillus* (Scorpiones: Buthidae): Report of a rare event in scorpions

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Abstract. Leucism is a congenital disorder in which the individual is born with partial hypopigmentation. It is quite common in vertebrates, but rare in invertebrates, especially in arachnids like scorpions. This paper presents the first record of this congenital disorder to be observed in the order Scorpiones. During field studies in the Area de Conservação Aldeia-Beberibe, a set of Atlantic forest fragments of 31,634 hectares, we collected a pregnant leucistic female *Tityus pusillus* Pocock, 1893. In this female, the variegated pattern described for the species was a lighter color than normal. The animal produced 10 normal juveniles (not leucistics). In addition, we analyzed 1,164 specimens from 17 populations deposited in the CA-UFPE to verify the frequency of leucism; there were no scorpions with leucism within the analyzed populations. Thus, a break in variegated pattern, as with the leucism described in this study, may increase the mortality rate due to predation.

Key words: Brazilian Atlantic Forest, color pattern, depigmentation degree

Color patterns in scorpions have often attracted the attention of researchers, principally for ecological and taxonomical studies (Harington 1984; Lourenço & Cloudsley-Thompson 1996; Lourenço 2002; Vignoli et al. 2005; Olivero et al. 2012). Coloration patterns in these arachnids can vary from pale yellow to black, and the presence of sub-cuticular pigments form different configurations such as longitudinal or confluent bands (Lourenço 2002). Variations from these patterns are well documented for these arachnids, producing different morphology types ('morphs') for the same species (Lamoral 1979; Williams 1980; Harington 1984; Vignoli et al. 2005).

Environmental factors seem to play a role in many scorpions' color variations (Lourenço & Cloudsley-Thompson 1996; Lourenço 2002). For example, with the buthid scorpions *Tityus costatus* (Karsch, 1879) found in the southern portion of the Brazilian Atlantic forest, those that live at sea level (dry environment) are 'light morph,' whereas individuals that occur at 1000 m above sea level (wet environment) are 'darker morph' (Lourenço 2002). Olivero et al. (2012) also found color variation within six Argentinean populations of the bothriurid scorpion *Bothriurus bonarieusis* (C.L. Koch, 1842); these authors also reported that scorpions from wet sites are darker than specimens found in drier sites. Another form of variation in scorpion coloration is a complete absence of pigmentation, commonly found in animals that inhabit caves (Mitchell 1968, 1972; Francke 1977, 1978).

In some cases, however, atypical coloration can occur without environmental influences, due to an excess (melanism) or an absence (albinism) of color pigment in part or all of the body (Sanchez-Hernandez et al. 2012). Albinism is a very rare event in scorpions, being reported in the literature only for the Australian scorpion *Urodacus yaschenkoi* (Birula, 1903) (Locket 1986). This author reported the presence of two albino specimens from the south of Australia, and also compared the eye structure between normal and albino animals, finding abnormalities in the eyes of albino scorpions. Albinism is an extreme form of an absence of color pigmentation in the body, but some animals show a partial hypopigmentary congenital disorder called 'leucism' (Herreid & Davies 1960), which is very common in vertebrates (Sanchez-Hernandez et al. 2012) but not in invertebrates. Here, a case of leucism in the scorpion *Tityus pusillus* Pocock, 1893 is described, together with data on its offspring and the frequency of occurrence in the population.

This is a small ambush predator and the most common scorpion species in the northeast Brazilian Atlantic forest (Lourenço 2002; Lira et al. 2013; Lira & Albuquerque 2014). *Tityus pusillus* is typically found inhabiting the forest floor, with its abundance correlated with climatic conditions and microhabitat structure (Lira et al. 2013, 2015). The species possesses a yellow basal color and brownish variegated coloration (Lourenço & Cloudsley-Thompson 1996; Lourenço 2002).

Leucism in *T. pusillus* was recorded in a pregnant female collected during a nocturnal field study conducted in the Área de Preservação Ambiental Aldeia-Beberibe, a set of Atlantic forest fragments of 31,634 hectares (7° 54′ 48″S 35° 2′ 36′ W) (CPRH 2015). The leucistic individual was maintained in laboratory conditions and gave birth to 10 normal (non-leucistic) young. Confirmation of female gender was carried out according to Lourenço (2002), following death after the young dispersed from her dorsum. The specimen was then deposited in the Arachnological Collection of Universidade Federal de Pernambuco, Brazil.

Leucism (Fig. 1A) was characterized by a lighter scale of pigmentation of the variegated pattern of coloration common for the species in relation to the normal color range of individuals (Fig. 1B & C). To verify the frequency of leucism in this species, we examined 1,164 individuals from 17 different populations deposited in the Arachnological Collection (Table 1). Except for the leucistic female, no other record of leucism among individuals from the different populations analyzed was registered.

Scorpions' coloration constitutes the first line of defense of these animals, whose color patterns primarily have cryptic significance (Polis 1990; Lourenço & Cloudsley-Thompson 1996). These authors also suggest that most scorpion species that inhabit forests present two patterns of coloration, darker (darker brownish or black) and variegated (mottled). These colorations are camouflage within the darker environment found in forest interiors (Cloudsley-Thompson 1993a, b). Thus, the sedentary behavior shown by *T. pusillus* specimens (Lira et al. 2013) associated with variegated coloration may work as an effective defense against potential predators. Consequently, lighter morph types, such as the leucistic coloring described in the present study, would be more exposed to predation due to the lack of cryptic protection. In conclusion, our study describes for the first time the occurrence of leucism in scorpions; this event is rare, corresponding to a rate of just 0.06% in the population examined.

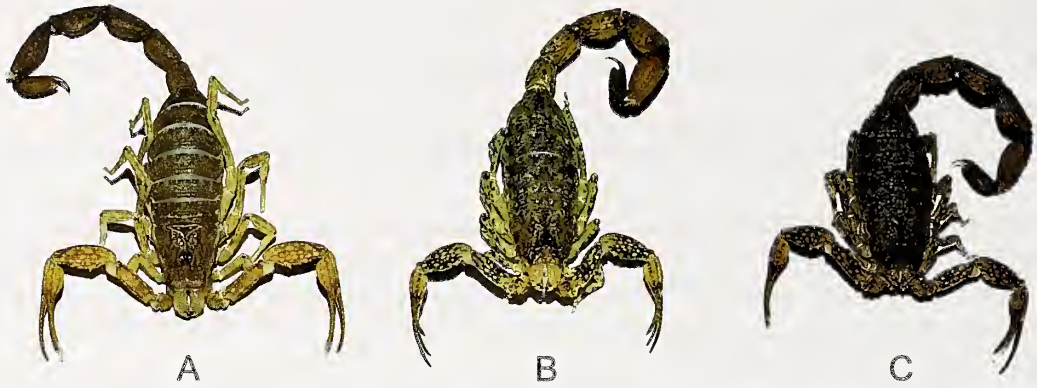

Figure 1.—Variation of color pattern of the scorpion, *Tityus pusillus* Pocock, 1893. A) Leucistic individual. B) Lighter individual. C) Normal individual.

Table 1.—Number of scorpions examined in the seventeen populations.

Location	Coordinates	Scorpions examined
Paudalho	7°54′48″S, 35°02′36″W	60
Água Preta	8°41′31.4″S, 35°29′49″W	2
Jaqueira	8°43′03.9″S, 35°50′21.6″W	104
Ipojuca	8°31′48″S, 35°01′05″W	24
Abreu e Lima	7°46′55″S, 35°09′02″W	101
Recife	8°00′00″S, 34°56′00″W	50
Tamandaré	8°43′43″S, 35°10′39.8″W	60
Moreno	8°06′38.1″S, 35°06′56.4″W	165
Timbaúba	7°36′36.6″S, 35°22′46.6″W	80
Igarassu	8°00′05.8″S, 34°52′23.1″W	85
Buíque	8°35′08.2″S, 37′14′29.3″W	48
Gravatá	8°11′11.8″S, 35°33′51.9″W	37
São Bento do Una	8°31′45.7″S, 36°27′23.8″W	6
Sirinhaém	8°38′59.0″S, 35°10′26.6″W	96
Jaboatão dos Guararapes	8°02′34″S, 35°02′22″W	86
Rio Formoso	8°32′07.6″S, 35°05′53″W	150
Caruaru	8°22′09″S, 36°05′00″W	10

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SHORT COMMUNICATION

Egg sac parasitism: how important are parasitoids in the range expansion of the wasp spider *Argiope bruennichi*?

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Abstract. During recent decades, the wasp spider, *Argiope bruennichi* (Scopoli, 1772), has expanded relatively quickly towards north Europe. As a consequence of its spreading, it is newly exposed to various factors of selection. We studied the impact of egg sac parasitoids on the mortality of *A. bruennichi* in three regions differing in climate conditions and time of settling by this spider. Parasitism of wasp spider egg sacs was relatively low (0–3.9%) and no significant differences between studied regions were found. One primary parasitoid, *Tromatobia ornata,* was reared; in approximately 60% of these parasitized cocoons, the entire content of the egg sac was destroyed.

Keywords: Pseudohyperparasitoid, host, *Tromatobia ornata, Pediobius brachycerus*

The wasp spider, *Argiope bruennichi* (Scopoli, 1772) is a Palaearctic species that has been expanding northward from the Mediterranean (Kumschick et al. 2011); its European range currently also includes Scandinavia (Jonsson & Wilander 1999; Bratli & Hansen 2004; Koponen et al. 2007). In Poland, the wasp spider was first recorded in 1935 (Urbański 1935), and, for several decades, its area was restricted only to the west and south-eastern parts of the country. Since the 1990s, *A. bruennichi* has rapidly spread towards the north. Now, the wasp spider occurs throughout Poland and is numerous everywhere, except for the higher elevation of the mountains (Wawer 2012). The expansion of some European species to the north may be connected with global warming (e.g., Hughes 2000; Walther et al. 2002; Hickling et al. 2006). In the case of the thermophilous wasp spider, this may be one of the most important factors (Ivinskis et al. 2009), but other factors may also favor its spreading, e.g., prolongation of the growing season, increasing the area of fallow lands or transport intensification causing passive dispersion (Guttmann 1979).

As a consequence of expansion, *A. bruennichi* is exposed to new factors of selection (Leborgne & Pasquet 2005). Natural enemies have a significant mortality impact on local spider populations (Polis et al. 1998), and parasitoids might be the most important of these (Foelix 2011). Among parasitoids, there are some that develop individually within eggs (e.g., Scelonidae), or feed on spider egg masses (e.g., Mantispidae, Ichneumonidae, Phoridae), as well as ectoparasitoids (e.g., Ichneumonidae) and endoparasitoids (e.g., Acroceridae) of post-embryonic spiders—both spiderlings and mature spiders (Austin 1985; Fitton et al. 1987; Schlinger 1993; Allard & Robertson 2003; Finch 2005). The mortality of eggs and spiderlings is considered to be the most significant factor regarding the spider life cycle (Topping 1997). Parasitoids that are associated with the wasp spider are species of the Ichneumonidae and Eulophidae (Hymenoptera). Among the ichneumonid wasps, *Tromatobia ornata* (Gravenhorst, 1829) from the Pimplinae (Rollard 1985, 1990) and *Buathra tarsoleucos* (Schrank, 1781) and *Thaumatogelis gallicus* (Seyrig, 1928) from the Cryptinae have been recorded as parasitizing *A. bruennichi* (Fahringer 1922; Schwarz 2001). These species develop by feeding on cocooned spider eggs, but none of them are specific to *A. bruennichi* (Fitton et al. 1987; Yu et al. 2012). The parasitic wasp from the Eulophidae, *Pediobius brachycerus* (Thomson, 1878) is an obligatory hyperparasitoid (secondary parasitoid) of spider egg sacs, which necessarily parasitizes a spider's primary parasitoids, including some species of ichneumonid parasitoid wasps (Fitton et al. 1987; Kostro-Ambroziak & Wawer 2015). Here we studied the influence of egg sac parasitoids on the mortality of *A. bruennichi* in regions differing by time of settling of this spider and climatic conditions, based on populations from Poland.

Investigations were carried out from 2011 to 2013. Egg sacs of *A. bruennichi* were collected from nine localities in three regions of Poland: the Suwalki Lake District (SLD1: 54°8′14.88″N, 22°55′58.94″E; SLD2: 54°8′32.29″N, 22°50′55.37″E; SLD3: 54°5′25.23″N, 22°59′12.55″E), the Mazovian Lowland (ML1: 52°19′5.39″N, 20°52′32.29″E; ML2: 52°22′46.67″N, 20°47′47.04″E; ML3: 52°0′29.66″N, 21°22′12.58″E), and the Sandomierz Valley (SV1: 50°10′38.01″N, 21°43′12.09″E; SV2: 50°10′48.08″N, 21°42′31.02″E; SV3: 50°8′57.20″N, 21°40′36.67″E) (Fig. 1).

In south-east Poland, the first individuals of *A. bruennichi* were discovered in the 1960s (the Low Beskids) (Bednarz 1966). In the Mazovian Lowland, *A. bruennichi* was observed for the first time in 1998 (Kajak & Łuczak 2003). At that time, this species was considered to be rare and endangered in Poland, which led to its protection by law. In northern Poland (the Suwalki Lake District), the wasp spider was observed for the first time in 2005 (W. Wawer, unpubl.). Meanwhile, recent years have brought a wave of expansion of unusual intensity, causing species dispersal and establishment across virtually the entire country. The number of known locations doubled from 1990 to 2007 (Wawer 2014). The three regions mentioned above (SLD, ML, SV) differ in climatic conditions — the region furthest to the north is the coldest and probably due to this factor, it is characterized by fewer *A. bruennichi.*

Egg sacs overwintered in natural conditions on plant leaves, ca. 20 cm above the ground, in open areas, mainly in meadows and on agricultural wastelands. In April, they were collected and transported in a plastic jar and kept at a constant temperature of 8 °C until the dissection began. The egg sacs were opened at the end of April. Each egg sac was examined under a microscope and cut by medical scissors. Parasitized egg sacs were stored at room temperature on a piece of cotton wool in a plastic jar (50 ml), and every day, a few drops of water were added to preserve humidity. The adult parasitic wasps emerged after about 10 days (April/May).

Adult wasps were identified by morphological characteristics with reference to the taxonomic literature (Bouček 1965; Fitton et al. 1988). Additionally, pupal cases of parasitoids were confirmed by DNA barcode sequences. Reference Sequences (RefSeq) were obtained in this study. Genomic DNA was extracted using a Genomic

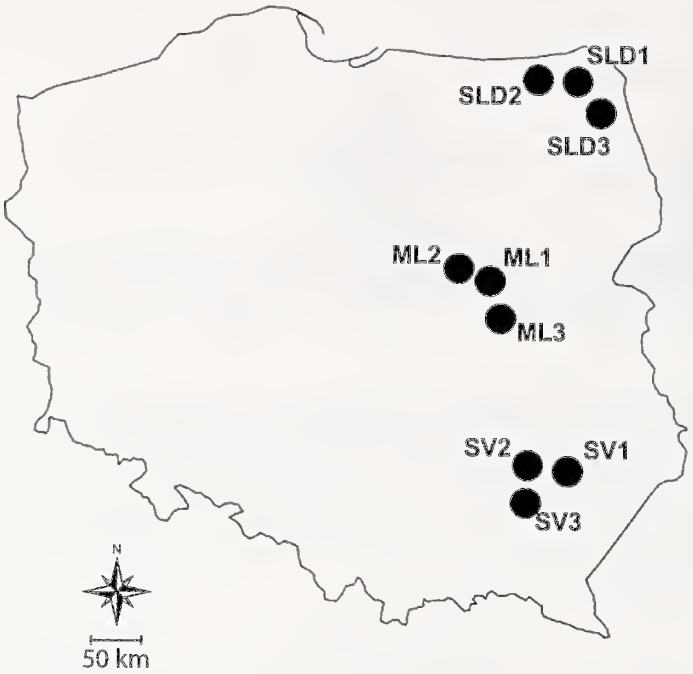

Figure 1.—Distribution of localities in Poland where the egg sacs of *Argiope bruennichi* were collected: the Suwalki Lake District (SLD), the Mazovian Lowland (ML) and the Sandomierz Valley (SV).

Mini Kit (A&A Biotechnology). The primers LepF1 (5′-ATTCAACCAATCATAAAGATATTGG-3′) and LepR1 (5-TAAACTTCTGGATGTCCAAAAAATCA-3′) were used for COI mtDNA fragment amplification (650 pz) (Smith et al. 2009). PCR consisted of an initial activation step at 95 °C for 15 min; 45 cycles of denaturation at 94°C for 30 sec, annealing at 52 °C for 90 sec and extension at 72 °C for 60 sec and a final extension at 60 °C for 30 min. The sequence data for the COI gene sequences were submitted to GenBank with the accession number KU870312. All voucher specimens are deposited in the collection of Museum and Institute of Zoology Polish Academy of Sciences.

In total, 560 egg sacs of *A. bruennichi* were examined. The degree of parasitism of wasp spider egg sacs was 3.9% (Table 1). In the central region ML, we noticed no infested egg sacs. No significant differences between the other two regions (SLD and SV) were found (Fisher's Exact test, $P = 0.21$). One primary parasitoid, *Tromatobia ornata*, was reared from 22 egg sacs and the secondary parasitoid *Pediobius brachycerus* from 11 egg sacs (Table 1). The former parasitoids were noticed as pupae (1–3 per sac) and the latter as larvae inside pupal casings (1–8 per sac). Only two egg sacs produced two or three individuals of *T. ornata*. The reproductive fitness of the *A. bruennichi* female was greater than zero in all cases of parasitization. In 41% of parasitized cocoons, an average of 148 living nymphs of spiders per egg sac were detected (SD = 100.1; range = 1–220). The unparasitized egg sacs either contained up to 642 living nymphs of the wasp spider, or were damaged, empty or with undeveloped embryos. Both in parasitized and unparasitized egg sacs, a portion of the spider eggs failed to develop (59% and 35.3%, respectively), a statistically significant difference (Chi-square test, $X^2_1 = 475.5$, $P < 0.001$).

The egg-sac of *A. bruennichi* is under maternal care for a few days (Leborgne & Pasquet 2005). The layer structure of the wasp spider cocoon protects eggs and spiderlings from fluctuating temperatures and desiccation, as well as acting as a mechanical barrier against parasitoids and parasites (Hieber 1985, 1992; Bergthaler 1995). *Tromatobia ornata* lays eggs into spider cocoons in reddish threads close to the outer layer (Rollard 1990). Parasitism of the egg sac of *A. bruennichi* by *T. ornata* in Poland is distinctly lower (0–3.9%) than in Germany (0–50%) (Sacher 2001) or France (8–44%) (Leborgne & Pasquet 2005), where *T. ornata* is also a main parasitoid in egg sacs of this spider species. The low degree of parasitism in Poland may be the effect of a shift in the time of oviposition of host and parasitoid caused by the recent spread of *A. bruennichi*. Rollard (1987) revealed that egg sacs were parasitized by *T. ornata* only by the time juveniles emerged. The spider embryos develop in approximately 2 to 3 weeks and nymphs hibernate during winter in the egg sac (Von Becker 1983; Rollard 1987). Because of this, females of *A. bruennichi* that lay their eggs early avoid parasitoids and have higher reproductive success (Leborgne & Pasquet 2005). Because *T. ornata* is relatively widespread in Poland, it is probable that here it parasitizes other hosts, both other spiders and moths (Yu et al. 2012), and the wasp spider is not a limiting factor for this parasitoid.

Argiope bruennichi lays on average 800 eggs per sac (Rollard 1985; Köhler & Schäller 1987; Miyashita 1996). Larvae of *T. ornata* feed on spider eggs and develop very quickly to fifth instars, and in this inactive stage they overwinter inside the spiders' egg sacs (Rollard 1985). Little is known about the phenology of this parasitoid from spring to autumn. Oehlke & Sacher (1991) indicated that *T. ornata* is univoltine, but based on the biology of its other hosts (Yu et al. 2012), e.g., *Nuctenea umbratica* (Clerck, 1757), it is highly probable that it is at least bivoltine. Although *T. ornata* is known as a gregarious parasitoid (Fitton et al. 1988; Sacher 1988, 2001), we recorded mainly single specimens of it in egg sacs. We also noticed that not all of the parasitized egg sacs were destroyed. Rollard (1985) indicated that total destruction of spider eggs occurred only when there was more than one larva of *T. ornata* in a cocoon. According to Cortés et al. (2000), *Tromatobia* sp., as a parasitoid of *Araneus granadensis* (Keyserling, 1864), also destroys only a portion of the contents of the egg sac.

We recorded *P. brachycerus* as a gregarious parasitoid inside the pupae of *T. ornata*. According to these data and the earlier suggestion of Fitton et al. (1987) that *P. brachycerus* attacks the primary parasitoid during the pupal stage, we think it should be labelled as a pseudohyperparasitoid. In contrast to hyperparasitoids, which parasitize the larvae of other parasitoids while they are feeding on or in the primary host, pseudohyperparasitoids attack the primary parasitoid after it has completed feeding on its host (Quicke 2015). Of course, detailed studies on the biology of this parasitoid wasp are needed. *Pediobius brachycerus* is a parasitoid of some species of Ichneumonidae which parasitize spider eggs (Kostro-Ambroziak &

Table 1.—Egg sac parasitism of *Argiope bruennichi* in three regions differing in climate conditions and time of settling by the spiders: the Suwalki Lake District (SLD), the Mazovian Lowland (ML) and the Sandomierz Valley (SV).

Region	N of studied egg sacs	N and (%) of egg sacs parasitized by *T. ornata*	N and (%) of *T. ornata* pupae parasited by *P. brachycerus*
Suwalki Lake District (SLD)	220	15 (6.8%)	6 (33.3%)
Mazovian Lowland (ML)	220	0	0
Sandomierz Valley (SV)	120	7 (5.8%)	6 (75%)
Total	560	22 (3.9%)	12 (46.15%)

Wawer 2015), but *T. ornata* is recorded for the first time as its secondary host.

To summarize, in our study the overall mortality of *A. bruennichi* induced by its egg parasitoids was relatively low. Additionally, a high level of undeveloped eggs both in the parasitized and unparasitized egg sacs suggests that other factors, such as unfavorable wintering conditions (temperature, humidity) or fungal penetration, may have a significant impact on the mortality and life history of this range expanding spider.

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SHORT COMMUNICATION

Endosymbiotic Rickettsiales (Alphaproteobacteria) from the spider genus *Amaurobioides* (Araneae: Anyphaenidae)

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Abstract. Endosymbiotic bacteria are commonly found in terrestrial arthropods and their effects have been studied extensively. Here we present the first recorded case of endosymbiotic bacteria found in the spider family Anyphaenidae. A fragment of the cytochrome oxidase c subunit I "barcoding" region belonging to unidentified Rickettsiales, presumably belonging to the genus *Rickettsia*, was sequenced from six individuals of *Amaurobioides africana* Hewitt, 1917.

Keywords: Bacteria, barcoding, intertidal

The gram-negative proteobacteria are one of the most widespread and diverse groups of bacteria, including medically important pathogens, free-living nitrogen-fixing organisms, and the order Rickettsiales, which contains obligate intracellular bacteria such as *Wolbachia* and *Rickettsia* that can be found living inside the cells of terrestrial arthropods (Ferla et al. 2013). These two genera have been the subject of numerous studies in arachnids, as they represent important pathogens in some cases (e.g., Paddock et al. 2010), while in others they are endosymbionts that manipulate their host's physiology, behavior and/or bias the host's sex ratio to favor their transmission (Rowley et al. 2004; Goodacre et al. 2006; Duron et al. 2008; Gunnarsson et al. 2009; Wang et al 2010; Vanthournout et al. 2011; Goodacre & Martin 2012, 2013). *Rickettsia*-infected spiders have also been shown to display increased long-distance dispersal tendencies (Goodacre et al. 2009). Bacterial endosymbionts are usually transferred vertically in spiders, although there is evidence for horizontal transfer in closely related taxa (Baldo et al. 2008).

Early methods of detection of bacterial endosymbionts in insects and spiders relied on staining techniques (Cowdry 1923). With the advent of PCR-sequencing techniques, molecular detection of specific endosymbionts was made possible with relative ease, in the case of spiders resulting in targeted studies (e.g., Baldo et al. 2008; Jin et al. 2013) or sometimes as a byproduct of a study with a different aim (e.g., Řezáč et al. 2014 in *Dysdera microdonta* Gasparo, 2014). With bacteria-specific primers, amplification of endosymbiont DNA from potential host tissue provides positive results only for infected hosts. On the other hand, more "universal" primers may find the annealing sites in both host and symbiont, if said sites are conserved enough for the genomic region. One such case appears to be the "barcoding" fragment of the Cytochrome Oxidase C subunit I (COI), a protein-coding gene that appears to have its origins deep within the origins of life on the planet (Castresana et al. 1994). The fact that COI has highly conserved regions means that certain primers can be used across a wide range of organisms to amplify the same gene region. This is advantageous if the tissue used for DNA extraction exclusively belongs to one species. However, in the case of organisms hosting endosymbionts, this could be seen as a complication, although for large-scale barcoding studies, it has been shown to be manageable (Smith et al. 2012).

As part of a larger study on the intertidal anyphaenid genus *Amaurobioides* O. Pickard-Cambridge, 1883 (Araneae: Anyphaenidae) (Ceccarelli et al. in prep.), DNA was extracted from leg tissue of 19 individuals of *A. africana* Hewitt, 1917 and approximately 630 base-pairs of the COI gene fragment were amplified and sequenced using the primers LCOI 1490 (Folmer et al. 1994) and HCOoutout (Prendini et al. 2005). For six out of the 19 individuals, the sequenced COI region did not belong to the targeted host species, but to an unknown species of the order Rickettsiales, presumed to be an intracellular symbiont. There was no variation in the nucleotides of the six sequences obtained, indicating that the *A. africana* individuals in this study were all infected with the same bacterial species. The sampling localities of the six infected specimens are shown in Fig. 1a and the COI sequences have been deposited in GenBank (accession numbers KU600819–KU600824).

The identification of the Rickettisales COI sequences amplified in this study was based on comparisons to sequences available in the public databases *International Barcode of Life Database* (BOLD systems; http://www.boldsystems.org/) and *GenBank* (http://www.ncbi.nlm.nih.gov/genbank/). The information available from BOLD was minimal (the level of identification provided was to the order Rickettsiales) and there were cases of misidentification in GenBank, as the *BLAST*-queried sequences from this study were 99% identical to COI sequences labelled as "Hymenoptera sp.", while the correct identification as proteobacteria started at 90% identity.

At this point, questions relating to why non-bacterial primers preferentially amplified COI regions of endosymbionts rather than host DNA in this study—even resulting in clean sequences (rather than a mix of host and symbiont amplicon)—remain largely unanswered. A visual inspection of the priming sites revealed that 9 Rickettsiales COI sequences downloaded from GenBank had 80–90% identity in the last 10 base-pairs towards the 3′ end of the forward and reverse primers. This base-pair identity, coupled with the possibility of a very high number of endosymbiotic Rickettsiales, may have been enough to give initial preference and later exclusivity to the bacterial over the host DNA for primer annealing and DNA amplification during PCR. As mentioned earlier, the presence of endosymbionts is not thought to interfere with DNA barcoding of arthropods when using universal primers (Smith et al. 2012). However, the possibility still exists that COI sequences of endosymbionts are obtained when in fact the target organism is the host, as shown in this study, along with other isolated cases (e.g., Řezáč et al. 2014) and the presence of misidentified Rickettsiales COI sequences in GenBank (where the target organism was the host and thus the identification was placed as Hymenoptera sp.).

Of the COI sequences in GenBank from Rickettsiales (all belonging to the genus *Rickettsia*) with an identity score >75% for the sequences from this study, ten were selected for Bayesian phylogenetic analyses, along with a sequence from a closely related genus (*Orientia*, based on Weinert et al. 2009), four sequences of *Wolbachia* and a sequence belonging to *Anaplasma*, to root the tree. The COI sequences were

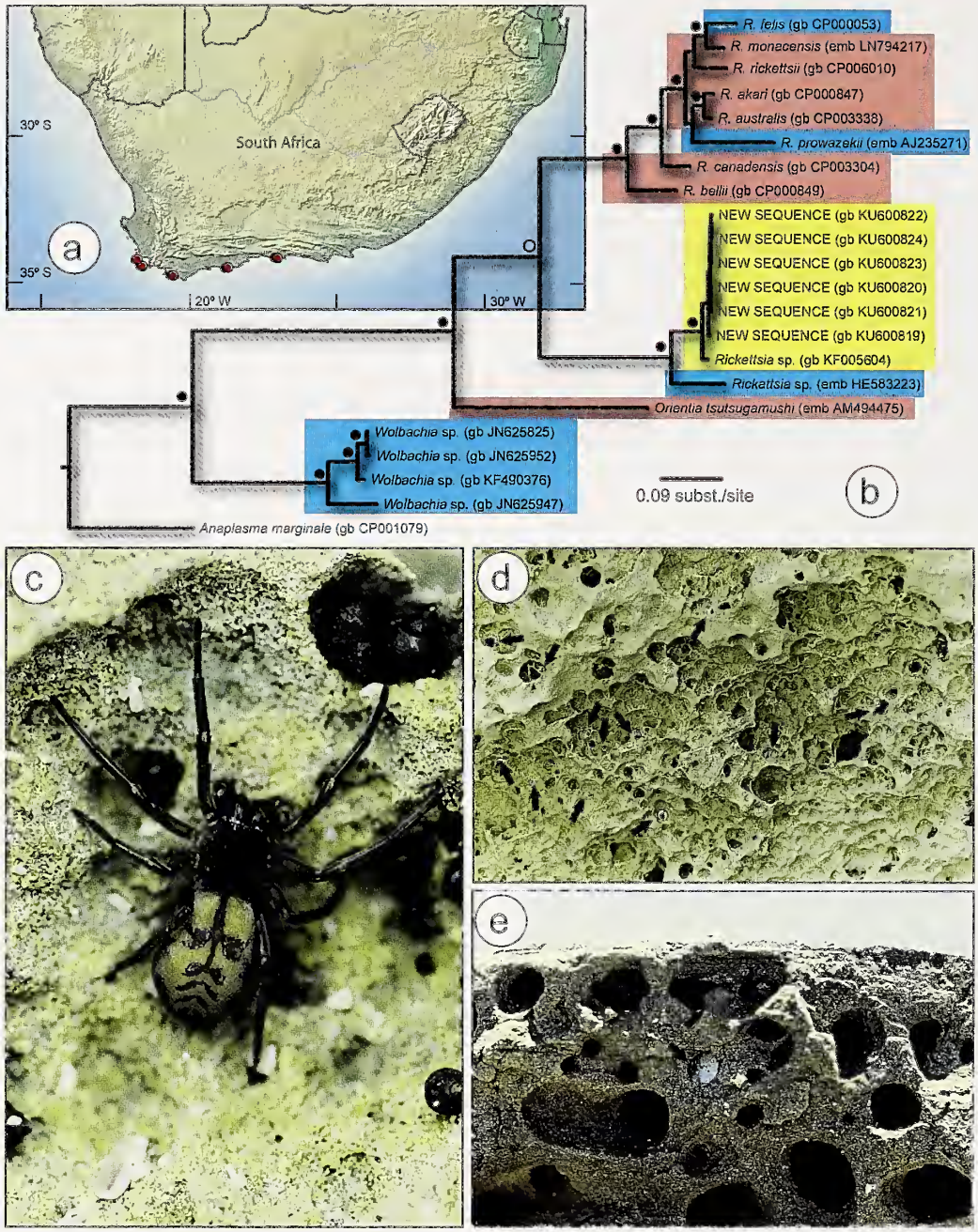

Figure 1.—a. Map showing localities where Rickettsiales-infected specimens of *Amaurobioides africana* were collected; b. Bayesian phylogenetic tree of COI sequences for selected *Rickettsia* species and specimens from closely related genera, obtained from the NCBI database. Nodal support in Bayesian posterior probability (PP) represented by filled (0.95 < PP <= 1) and empty (0.9 < PP <= 1) circles. GenBank accession numbers are shown after taxon names in brackets. Terminal taxa labelled as NEW SEQUENCE are from this study. Colored boxes around taxon names represent host classes (blue = Insecta; red and yellow = Arachnida; arachnid orders: red = Acari; yellow = Araneae); c. General habitus of *A. africana*; d–e. Rock faces in the intertidal zone at De Hoop Nature Reserve, showing abandoned retreats (arrows) of *A. africana* (d), and at Jeffrey's Bay, showing sealed retreats of *A. africana* (e). Photos: C.R. Haddad.

aligned using TranslatorX (Abascal et al. 2010) and a partitioning strategy along with nucleotide substitution models for each partition (TrNef+I, TrN+G and TrN+I+G for COI 1st, 2nd and 3rd codon positions, respectively) chosen by PartitionFinder v.1.1.1 (Lanfear et al. 2012). A Bayesian phylogenetic tree was obtained by forming a consensus of 20,000 trees (minus 10% burn-in) from 20 million generations of Markov Chain Monte Carlo simulations performed in MrBayes v. 3.2.3 (Ronquist et al. 2012). Based on the phylogenetic tree obtained (Fig. 1b), the sequences from this study belong to an unidentified *Rickettsia* species, closely related to a *Rickettsia* species infecting the spider *Dysdera microdonta*. Apart from being confident that *A. africana* can harbor the endosymbiont *Rickettsia*, a more in-depth study is required to fully understand the distribution, ecology and biology of the endosymbionts detected in this study.

Of particular interest in this relationship is the biology of the host spiders, which are exclusively found in the intertidal zone of rocky shores in marine habitats (Fig. 1c). The spiders regularly construct their silken retreats in rock faces (Fig. 1d, e), which they seal with silk

during high tide to avoid immersion in salt water, emerging at low tide to forage (Lamoral 1968). Therefore, transmission of the symbionts through the water medium in which the spiders occur seems unlikely. Apart from the most likely transmission pathway of the endosymbionts in *A. africana* being vertical transmission, the possibility of horizontal transmission should not be ruled out at this stage; a plausible additional explanation may be the transmission of the endosymbionts during ingestion of prey tissues, such as isopods, amphipods and dipterans that occur in the intertidal zone (Lamoral 1968). Further, the possibility that the same endosymbionts may infect various other spiders and pseudoscorpions occurring in the intertidal zone in South Africa (Lamoral 1968; Haddad & Dippenaar-Schoeman 2009; Larsen 2012; Owen et al. 2014), and the platygastrid wasp egg parasitoid of the only other truly exclusive intertidal spider in South Africa, *Desis formidabilis* (O. Pickard-Cambridge, 1890) (Desidae), viz. *Echthrodesis lamorali* Masner, 1968 (Owen et al. 2014), requires further investigation. Nevertheless, this study represents the first record of Rickettsiales in anyphaenids and is a contribution towards a broader understanding of proteobacteria in spiders.

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